ISSN 0377- 9335

ENTOMON

Volume 47 DECEMBER 2022 Number 4

46 YEARS OF EXCELLENCE

ASSOCIATION FOR ADVANCEMENT OF ENTOMOLOGY

ENTOMON

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https://doi.org/10.33307/entomon.v47i4.807

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ENTOMON 47(4): 353-364 (2022)
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Effect of stingless bee propolis on the proliferation of human pluripotent stem cells

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ABSTRACT: The effect of stingless bee propolis on the proliferation and differentiation of human stem cells is reported for the first time. Cells (hPSCs) treated with the propolis extracted from *Lisotrigona* sp., *Tetragonula calophyllae* and *T. travancorica* displayed a remarkable difference in their morphology. Gene expression analysis revealed pluripotency markers *OCT4* and *NANOG* to be down-regulated upon treatment with propolis, which confirmed early differentiation of hPSCs. Further investigation on the gene expression of early differentiation markers revealed that propolis supports mesendoderm differentiation, which is a novel finding. The propolis obtained from stingless bees *Tetragonula* spp. probably has more therapeutic value in terms of its effect on hPSCs viz., more tendency of the cells to differentiate into mesoderm and endoderm lineages, compared to the propolis obtained from *Lisotrigona* sp.

KEY WORDS: Gene expression analysis, therapeutic value, hPSCs, cytotoxicity, differentiation

INTRODUCTION

Stingless bees commonly called meliponines are a large group of bees, which belongs to the tribe Meliponini that is widely occurring over the tropical and subtropical areas of the world (Velikova *et al.*, 2000). Stingless bees are amongst the longest evolved bees that have been identified in 80 million years old parts of amber, estimated to have 400 to 500 different species but new species are identified every year (Kasote *et al.*, 2019). Three new species of stingless bees *Tetragonula travancorica*, *T. calophyllae* and *T. perlucipinnae* were described as new to science from Kerala (Shanas and Faseeh, 2019). Stingless bees use their head gland secretions, plant resins, wax, essential oils, pollen and exudates, including organic and inorganic earth components to produce propolis (Ghisalberti., 1979; Pasupuleti *et al.*, 2017). The colour of propolis varies from yellow to dark brown based on the origin of the resin. Propolis is known for its antibacterial, antifungal, antiviral, anti-

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inflammatory, antioxidant, anti-tumoral and tissue generation activities (Bankova and Popova, 2007; Popova *et al.*, 2019). Propolis exhibits a complex chemical composition and has been reported to contain more than 300 organic and inorganic compounds (Huang *et al.,* 2014). The chemical composition and pharmacological activities of propolis vary according to the geographical and botanical origin, types of vegetable sources, time of collection and season of the year (Wagh, 2013; Farooqui, 2012; Anjum *et al.*, 2011). India, being a vast country, has several different varieties of propolis varying in its chemical compositions and medicinal values. Moreover, the unique geography of Kerala being encroached upon by the Western Ghats provides a variety of propolis differing in chemical composition and medicinal values.

Stem cells are cells that have the potential to develop into different cell types in the body during early life and growth. Induced pluripotent stem cells (iPSCs) are the cells that are reprogrammed from somatic cells to form undifferentiated stem cells having the same properties as Embryonic Stem Cells (ESCs). ESCs are derived from early pre-implantation blastocyst stage embryos, that can self-renew indefinitely in culture and are pluripotent, maintaining the ability to become any cell type in the human body (Takahashi and Yamanaka, 2016). Human PSCs, including hESCs and hiPSCs, hold great promise for drug discovery and regenerative medicine as they can be used for disease modelling, drug screening and understanding of the mechanisms underlying development of tissues and organs (Wu and Hochedlinger, 2011; Robinton and Daley, 2012).

Natural compounds serve as a promising source of alternative medicine for various degenerative diseases. They can recruit stem cells, increase their proliferation and promote their differentiation. Several natural compounds and their combinations can promote the proliferation and differentiation of iPSCs in vitro (Bickford *et al.*, 2006). Propolis, a natural compound increased the proliferation rate of bone marrow-derived mesenchymal stem cells (BMMSCs), enhanced the chondrogenic and adipogenic differentiation processes. They also increased the migration capacity of BMMSCs and promoted induced gap closure of cells after osteogenic differentiation in vitro (Elkhenany *et al.*, 2019). Another study by using Taiwanese green propolis (TGP) ethanol extract promoted the differentiation of murine mesenchymal stem cells into adipocytes by the activation of the PPARγ (adipogenic transcription factors) dependent pathway. There was also an increase in adiponectin and intracellular triglyceride level in the cells (Chen *et al.,* 2020). One of the important components of propolis extract, caffeic acid phenethyl ester (CAPE), can enhance in vitro expansion of blood derived hematopoietic stem cells (HSPCs) by the upregulation of the expression of genes such as SCF, HIF-1 α, and HO-1 (Liu *et al.*, 2014). CAPE was also shown to promote the proliferative capacity of hematopoietic stem cells derived from umbilical cord blood *in vitro* (Ahangari *et al.,* 2012). Studies reported that ethanolic extract of propolis can promote bone regeneration and induce hard tissue bridge formation in pulpotom. Moreover, propolis also displayed acceptable biocompatibility and enhanced the endodontic regeneration process (Elgendy and Fayyaa, 2017). *In vivo* studies in rats suggested that oral administration of propolis enhanced the healing of fractured femur and increased bone mineral density (Guney *et al.,* 2011). Moreover intraperitoneal injection of CAPE, a major component of propolis enhanced bone regeneration in the rat calvarial defect model (Ucan *et al.*, 2013).

The effect of stingless bee propolis on stem cells is not very well understood. To our knowledge, there are no reports in the literature that describes the effect of stingless bee propolis on the proliferation of human - induced pluripotent stem cells. In this study, we investigated the effect of propolis extracts at different concentrations towards proliferation, cytotoxicity and lineage - specific differentiation *in vitro*.

MATERIALS AND METHODS

Materials: Propolis samples were collected from managed hives of three different species of stingless bees, viz., *Lisotrigona* sp. (Kollam District) and *Tetragonula* spp. (Thiruvananthapuram District), Kerala. Raw propolis samples were scraped out

from the hives and stored inside refrigerator for further investigations. The samples were named P1, P2 and P3 for propolis collected from *Lisotrigona sp., T. calophyllae* and *T. travancorica* hives respectively for convenience of the study.

Preparation of propolis extract solution: The stock solution was prepared by macerating 3g propolis at room temperature and dissolving it in 10 ml of 95 per cent ethanol. The solution was then incubated at 70°C for 30 min followed by centrifugation at 8800 rpm at 5°C for 10 min. The supernatant was then maintained at 4°C to avoid degradation.

Human Pluripotent Stem Cells (hPSCs): The Human Embryonic Stem Cell (hESCs) (BJNhem19) line was procured from Dr. Maneesha Inamdar, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), India. The human induced Pluripotent Stem Cell (hiPSCs) (D14C2) line, was a kind gift from Dr. R.V. Shaji, Centre for Stem Cell Research, (CSCR) – InStem, India.

The hiPSCs are maintained on Vitronectin (Gibco, A14700) and Essential 8 (E8) medium (ThermoFisher Scientific, A1517001). When the colonies become mature, they are seeded for the treatments. For the MTT assay, the hPSCs are seeded as single cells using Accutase in a Matrigel (Corning, 356234)-coated 96 well plate, at 300 cells/ well density in E8 medium with ROCK inhibitor (ROCK*i*), Y-27632 (Pepro Tech, 1293823) (10 µM). For propolis treatments, the cells are passaged using 0.5mM EDTA (Thermo, life technologies, 15575- 020) and seeded on Matrigel-coated 6-well plates in E8 medium.

For the MTT assay, StemPro Accutase (Thermo Fisher, A1110501) is used for cell dissociation into single cells and seeded with E8 medium supplemented with ROCK*i* on Matrigel-coated 96-well plates (300 cells/well) after counting the viable cells using Trypan blue. After 24 hours, ROCK*i* was withdrawn and the medium is replaced every day with fresh E8 medium. When the cells grown to small colonies, the media was replaced with N2B27 media supplimented with the three different propolis samples, 3LC (P1), TC (P2) and 5TT (P3) with different concentrations (150, 300, 450, 600 and 900µg ml^{-1}) and incubated at 37°C for 24 hours in a CO_2 incubator at 5 per cent CO_2. Next day, MTT assay is carried out using the CellTiter 96 Non-Radioactive Cell Proliferation Assay (MTT) kit (Promega- G4000). The absorbance values are recorded at 570 nm wavelength using a plate reader, followed by the calculation of the IC-50 values for each Propolis sample. A reference wavelength of 630nm is used.

For the propolis treatments, the medium is aspirated and washed with DPBS. Then, the cells are incubated with 0.5 mM EDTA for 3-4 minutes at 37°C. After incubation, aspirate 0.5 mM EDTA and dissociate the cell in fresh E8 medium using 1 ml pipette. Seeded the cells on Matrigel-coated 6-well plates in E8 medium. The cells are incubated at 37^0C and 5 per cent CO_2 in a CO_2 incubator (Thermofischer Scientific). Medium is replaced every day with fresh E8 medium. When they are 50-60 per cent confluent, the cells were exposed to the required concentration of propolis (200 µg ml^{-1}) in N2B27 medium.

Trypan blue dye exclusion assay: Seeding density was determined by cell counting by trypan blue dye exclusion assay, for which, 20 µl of the cell suspension is taken in a microfuge tube, to which 30 µl of PBS and 50 µl of 0.4 per cent trypan blue solution are added (creating a dilution factor of 5). With a cover-slip in place, 10 µl of the trypan blue-cell suspension was transferred to the chamber on the hemocytometer. Viable cells are counted (non-viable cells stain blue, viable cells will remain opaque) in the four corner squares.

Cell viability assay: The cell viability test was carried out using 3- (4, 5- dimethyl thiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) assay. Five different concentrations of the propolis samples were (150, 300, 450, 600 and 900µg ml^{-1}) were taken for the treatment. Cells treated with 95 per cent ethanol and cells alone in the culture medium for blank correction (after MTT assay) are used as controls. After 24 hour treatments, MTT assay was performed using CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT) kit (Promega-G4000). 15µl of the Dye Solution is added to each

well and incubated at 37°C for 2 hours 15 minutes at 37°C and 5 per cent CO_2 for 24 hours in a CO_2 incubator. After incubation, 100µl of the solubilization solution/stop mix is added to each well. After 1 hour, the contents of the wells were mixed to get a uniformly coloured solution and absorbance is recorded at 570nm wavelength using a 96-well plate reader (PerkinElmer® EnSpire Multimode Plate Reader).

Gene expression study: Total RNA is isolated by QIAzol Lysis kit (QIAGEN, 79306) The isolated RNA is quantified using NanoDrop Spectrophotometer (ThermoFisher Scientific) and converted to cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad, 1708891). Quantitative real-time PCR (qRT-PCR) is performed using the PowerUp™ SYBR™ Green Master Mix (2X) (Applied Biosystems, A25776) with gene-specific primers with Tm 58°C (Table 1) in a thermal cycler (Roche Light Cycler 480). The cDNA of control and treatment were subjected to qRT-PCR. Expression of nine genes (*NANOG, OCT 4, ACTB GSC, SOX17, SOX7, MSGN1, PAX6, NCAM1*) were studied. Data analysis is done using the *dd*Ct method, with the house-keeping genes, *GAPDH* or *ACTB*.

Table 1. List of primers and their sequences (5'- 3') used for qRT-PCR

Primer	Forward Primer (5'-3')	Reverse Primer (5'-3')
OCT4	GTGGAGGAAGCTGACAACAA	ATTCTCCAGGTTGCCTCTCA
NANOG	CCTGTGATTTGTGGGCCTG	GACAGTCTCCGTGTGAGGCAT
ACTB	TCAAGATCATTGCTCCTCCTGAG	ACATCTGCTGGAAGGTGGACA
GSC	GAGGAGAAAGTGGAGGTCTGG	CGACGTCTTGTTCCACTTCTC
SOX17	ACGTGTACTACGGCGCGATG	CTGGTGCTGGTGCTGGTGTT
MSGN1	CTGCACACCCTCCGGAATT	CTCTGCCGCGGTTAAGGAG
PAX6	CCAGGGCAATCGGTGGTAGT	ACGGGCACTCCCGCTTATAC
NCAM1	TCATGTGCATTGCGGTCAAC	ACGATGGGCTCCTTGGACTC
SOX7	TGCCCACTTCATGCAACTCC	AGGTACCCTGGGTCTTTGGTCA

Supplemental methods

1. MTT Assay

a) Cell plating

The human induced Pluripotent Stem Cells, D14C2 are seeded into 96 well plates.

ROCK inhibition:

- Aspirate the medium from the culture dish with 60 - 70 per cent confluent cells and wash with 1ml DPBS.
- Add 2ml of fresh E8 medium (ThermoFisher Scientific, A1517001) with 10 µM of ROCK inhibitor (ROCKi) (Y-27632, Peprotech – SM-1293823-B).
- Incubate for 1 hour at 37^{0}C and 5 per cent CO_2 in a CO_2 incubator (ThermoFischer Scientific).

➢ **Cell dissociation:**

- Aspirate the ROCKi containing medium from the culture dish and wash with 1ml DPBS (without Ca and Mg).
- Add 1ml of StemPro Accutase (Thermo fisher- A1110501) and incubate at 37^{0}C for 25 minutes.
- After incubation, add 1ml of E8 medium

with ROCKi into the dish and gently pipette up and down until cells are in a single cell suspension.

- Transfer the cell suspension to a 15 mL conical tube with 4 ml of E8 medium with ROCKi and centrifuge at 200 xg for 5 minutes.
- Aspirate the supernatant and re-suspend the cells in fresh E8 medium with ROCKi.
- Take a 20 µL sample of the cell suspension to determine viable cells.
- Plate the appropriate number of cells on Matrigel (Corning, 356234)-coated dish(es) and incubate at 37°C and 5 per cent CO_2 in a CO_2 incubator.

➢ **Cell Counting:**

- Transfer 20 µl of the cell suspension into a 0.5 ml microfuge tube.
- Add 30 µl of PBS and 50 µl of 0.4 per cent trypan blue solution to the cell suspension (dilution factor of 5) in the centrifuge tube.
- Mix thoroughly and incubate for 5 minutes.
- With a cover-slip placed on the chamber on the hemocytometer, transfer 10 µl of the trypan blue-cell suspension to the chamber (by carefully touching the edge of the cover-slip with the pipette tip and allowing the chamber to fill by capillary action).
- Count the viable cells (non-viable cells stain blue, viable cells will remain opaque) in the four corner squares.
- Calculate the total number of cells per ml

Cells per ml = the average count per square x the dilution factor x 10^4

$= (111/4) \times 5 \times 10^4$

$= 138.75 \times 10^4$

Seeding Density = 300 cells per well of 96 well plate

Cells taken per well = $300 / 138.75 \times 10^4 = 0.2$ µl

Therefore, 0.2 µl of D14C2 cells to each well of 96 well plate.

Propolis Treatments for MTT assay

When the cells are 70-80 per cent confluent, the cells are treated with the 3 different propolis samples, 3LC (P1), TC (P2) and 5TT (P3) at different concentrations (150, 300, 450, 600 and 900 µg ml^{-1}) in N2B27 medium. Cells treated with 95 per cent ethanol and cells alone in the culture medium for blank correction (after MTT assay) are used as controls. Incubate the plate at 37°C for 24 hours in a CO_2 incubator at 5 per cent CO_2.

b) MTT Assay

After 24 hour of propolis treatment, MTT assay is carried out using the CellTiter 96 Non-Radioactive Cell Proliferation Assay (MTT) kit (Promega-G4000). Add 15µl of the Dye Solution to each well. Incubate the plate at 37°C for 2 hours and 15 minutes at 37°C in a CO_2 incubator with 5 per cent CO_2. After incubation, add 100µl of the Solubilization Solution/Stop Mix to each well. Incubate for 1 hour at room temperature. After one hour, the contents of the wells may be mixed to get a uniformly coloured solution. However, care should be taken to avoid bubble formation. Bubbles on the surface may interfere with the accurate recording of absorbance values. Record the absorbance at 570 nm wavelength using a 96-well plate reader (EnSpire Multimode Plate Reader). The use of a reference wavelength will reduce background contributed by cell debris, fingerprints and other non-specific absorbance. A reference wavelength of 630 nm is used. The absorbance values are recorded and cell viability and the IC-50 value for each Propolis sample are calculated.

2. Propolis treatments

Cells are passaged when the hPSCs reach 80-90 per cent confluence by a chemical method using EDTA. Tilt the plate and aspirate the medium and

wash the cells with 0.5 mM EDTA (Thermo, life technologies, Cat. No. 15575- 020) in DPBS (Thermo, life technologies, Cat. No. 14190136). Aspirate the EDTA add 1 ml 0.5mM EDTA and incubate for 3-4 minutes at 37°C. EDTA is a chelating agent which functions in cell dissociation by blocking cell-cell adhesion by binding to Calcium and Magnesium ions on cell surfaces. Discard EDTA and gently flush the cells from the plate using E8 medium using a micropipette to dislodge the colonies. Make sure that the colony size is neither too big nor too small. Using a micropipette, transfer this solution to Matrigel (Corning, Cat. No- 356264)-coated 6 well plate and 3 cm dish (control) already containing E8 medium drop by drop. Observe under the microscope to ensure adequate colonies and appropriate colony size. Every day medium is replaced with fresh E8 medium.

When the cells reach 50-60 per cent confluence, the medium is aspirated and the wells are washed once with DPBS to remove any contents of E8 medium. The cells are treated with propolis (P1, P2 and P3) at a concentration 200 $\mu g\ ml^{-1}$ in N2B27 medium. The cells grown in N2B27 medium with FGF2 (20 $ng\ ml^{-1}$) or without ethanol are used as controls. After 24 hours, images are taken using the inverted microscope (Lawrence and Mayo phase contrast inverted microscopy) and the cells are lysed using a lysis buffer and used for RNA isolation (QIAzol Lysis kit: QIAGEN, 79306) and processed for qRT-PCR. The cell lysates and RNA samples may be stored in -80°C.

Matrigel Coating

1. Thaw Matrigel overnight by submerging the entire bottle in ice in a cold room or at 4°C. Use pre-chilled micropipette tips, serological pipettes, and tubes for diluting and aliquoting Matrigel.

2. The protein concentration of Matrigel varies across lots. Calculate the concentration of Matrigel required to coat and the appropriate volume of basal medium (dilution factor) accordingly. The final coating-concentration to be used is 8.7$\mu g\ cm^{-2}$. Once thawed, avoid freeze-thaw cycles. Aliquot working stocks and store at -20°C.

3. For coating the plates, add appropriate volume of ice-cold, serum-free basal medium (DMEM/F12 or DMEM) using a pre-chilled pipette tip to the fresh or frozen Matrigel aliquot. Gently mix by pipetting up and down, while the tube is on ice. Then transfer the diluted Matrigel to the center of the well (1ml/ well of a 6 well plate) and swirl gently to ensure a uniform coating.

4. For later use, wrap the Matrigel-coated plates tightly with parafilm to prevent drying up and store at 2-8°C for a maximum of one week. Prior to use, allow the coated plates to come to room temperature for about 1 hour.

5. For immediate use, incubate the plates at 37°C for an hour for gelation. Tilt the plate and aspirate-off the Matrigel solution. Add 1.5 ml E8 medium to each well and store at 37°C and 5 per cent CO_2 until cells are seeded into them.

Requirements for maintenance of hPSCs

- 0.5 M EDTA. pH 8.0 (Thermo, life technologies, Cat. No. 15575- 020)
- Essential – (E8) complete medium (Thermo, life technologies, Cat. No. A1517001)
- DPBS (Thermo, life technologies, Cat. No. 14190136)
- 6 – well plate (Eppendorf plate Cat. No 0030720016)
- 96-well plate (Nunc - Cat. No 161093)
- N2B27 media
  - DMEM/F12, (Thermo, life technologies, Cat. No. 11330032) - 46.6 ml
  - N-2 Supplement (100X), (Thermo, life technologies, Cat. No. 17502048)–0.5 ml
  - B-27 ®Supplement (50X), (Thermo, life technologies, Cat. No. 12587010)–1.0 ml
  - Bovine Albumin Fraction V (7.5 per cent soln., (Thermo Scientific, Cat. No. 15260037) – 340 µl

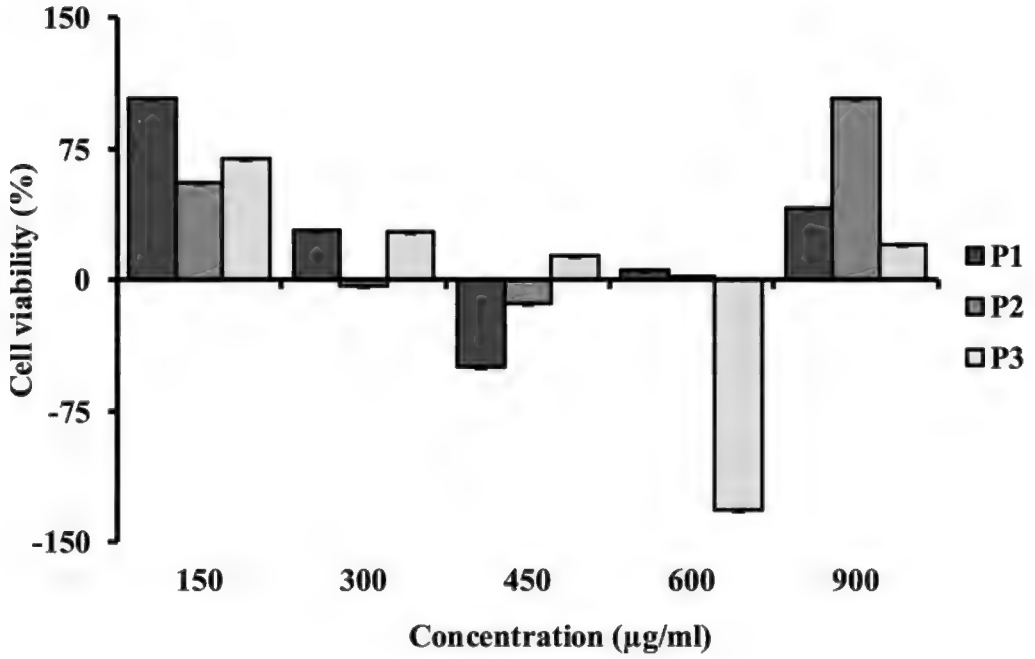

Fig. 1 Comparison of cell viability (MTT assay) of the cells treated with three propolis samples

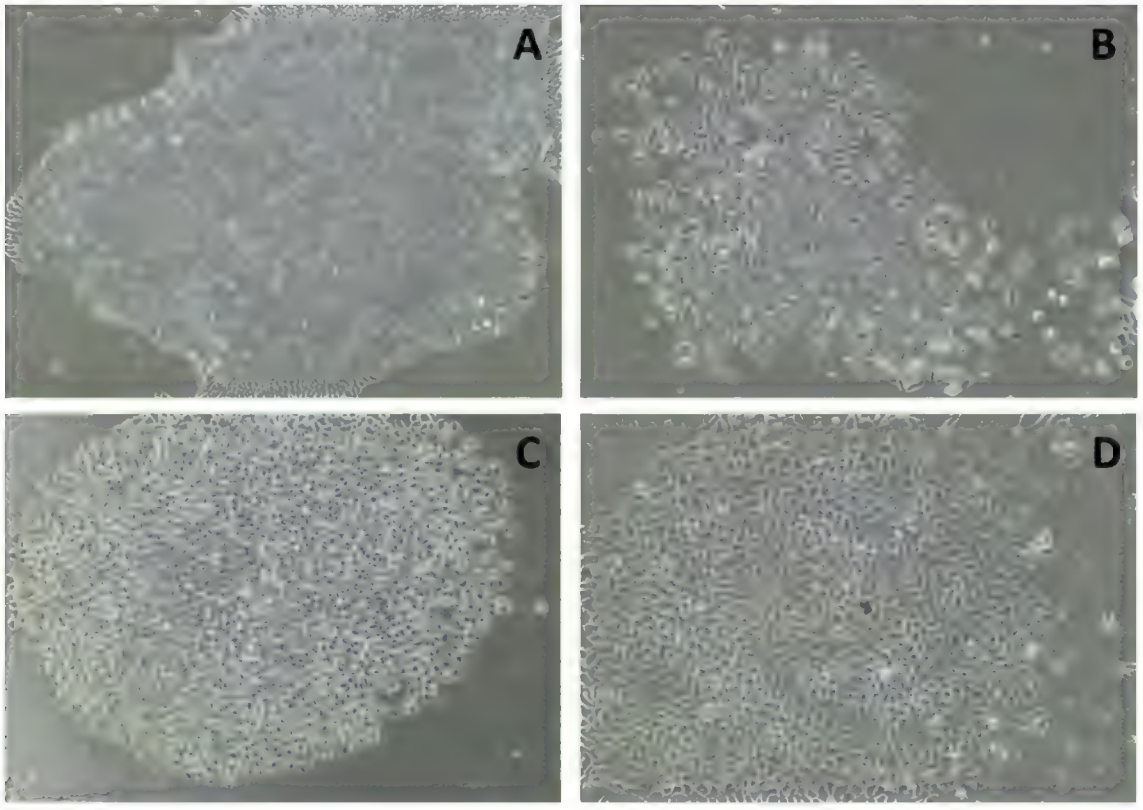

Fig. 2 Morphological changes in hiPSCs after treatment with propolis (A) Day 0 control, (B) P1, (C) P2, (D) P3

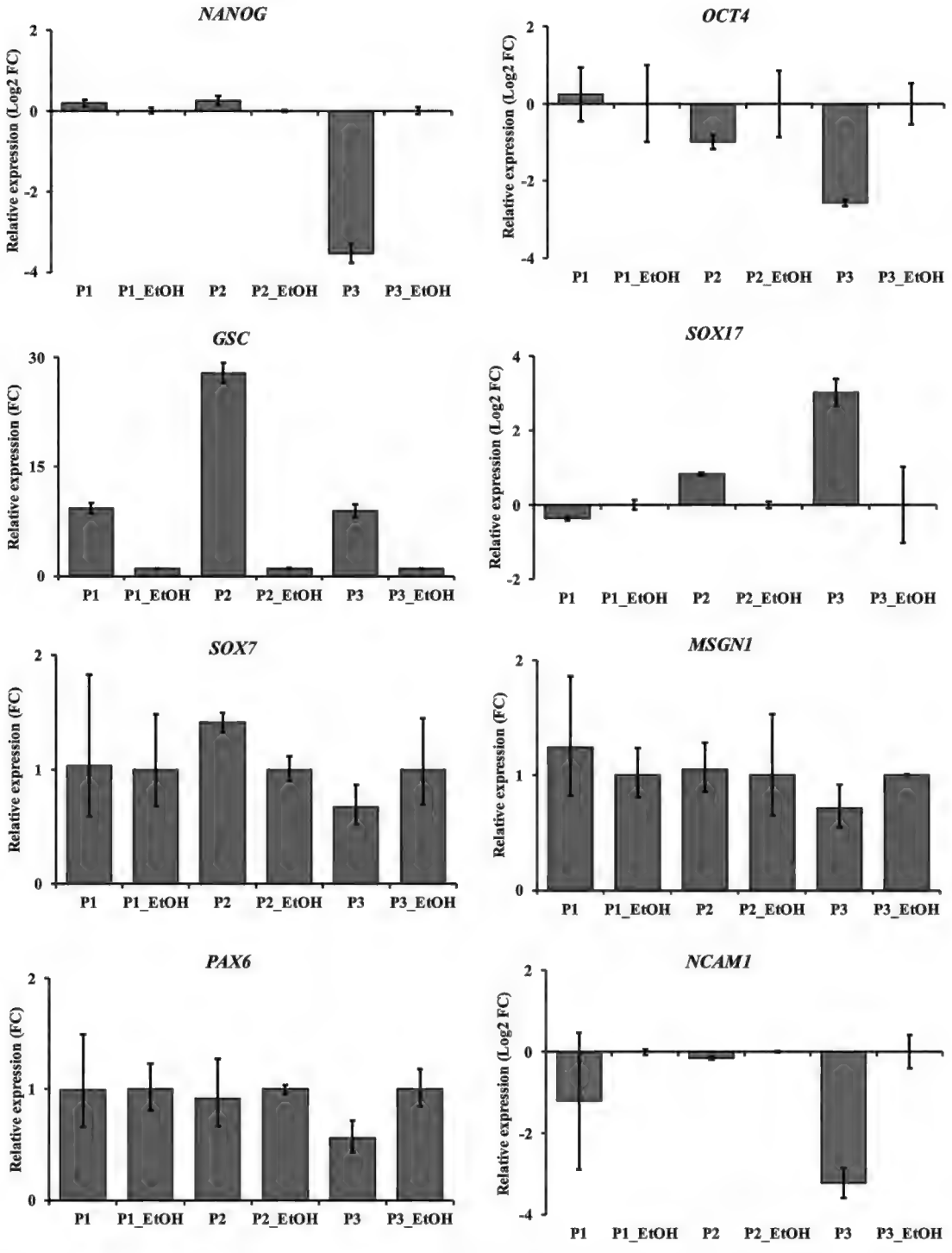

Fig. 3 Gene expression pattern of the mentioned markers after the hPSCs were treated with the propolis samples, P1, P2 and P3. ETOH: Ethanol control

- L-Glutamine (200mM), (Thermo Scientific, Cat. No. 25030149) – 0.5 ml
- Penicillin-Streptomycin, (Thermo Scientific, Cat. No. 15140122) – 0.5 ml
- â-mercaptoethanol, (Thermo, life technologies, Cat. No. 21985023) – 91.0 µl

RESULTS AND DISCUSSION

Propolis promotes cell proliferation and viability:

The influence of propolis on the proliferation and the IC-50 value of propolis was calculated by performing an MTT assay. Five different concentration of propolis - 150, 300, 450, 600 and 900 µg ml^{-1} were respectively added to D14C2 cells for a period of 24 hrs. Cells exposed to complete growth media without any propolis were used as normal control and cells treated with 95 per cent ethanol were taken as control. IC 50 value was determined for the three propolis samples. Cells treated with the propolis extracted from *Lisotrigona* sp. (P1), *T. calophyllae* (P2) and *T. travancorica* (P3) obtained IC50 values of 410.904, 480.097 and 215.157 µg ml^{-1} respectively. The cells displayed a significant proliferation rate after 24 h relative to the control however, higher concentrations were observed to be cytotoxic to cells (Fig. 1). When the cells were treated with propolis, the marked difference in the morphology of cells was observed. Hence it was confirmed that the propolis has an influences on early differentiation of hPSCs.

Morphological changes in hPSCs:

Induced pluripotent stem cells are usually observed as colonies with defined borders and shiny under the microscope. They are seen as a tightly packed cell with high nucleus to cytoplasm ratio, wherein the nucleus practically inhabits the entire cells. Cells when treated with crude propolis showed visible morphological changes compared with ethanol control indicating that propolis has some effect on these cells as they underwent spontaneous differentiation. When cells were treated with propolis, cells lost their border integrity, uniformity and started to migrate from the colonies (Fig. 2).

Early differentiation of pluripotent stem cells:

To study the differentiation potential of human pluripotent stem cells in the presence of propolis *in vitro*, gene expression of pluripotency and early differentiation markers were analysed (Fig. 3). Gene expression analysis revealed that the cells when treated with propolis, lost their pluripotent state. The transcription factors *NANOG* and *OCT4*, required for maintaining pluripotency displayed considerable downregulation in their expressions. Cells treated with propolis collected from the hives of *T. travancorica* showed very low expression of *NANOG* and *OCT4* compared to the other two propolis samples. These pluripotency markers are downregulated upon differentiation indicating that the propolis supported the hPSCs to differentiate. During differentiation, stem cells move into a transition state called primitive streak state or mesendoderm state. Goosicoid (*GSC*), a mesendoderm marker showed high expression in the cells which were treated with propolis. Among the three propolis samples, cells treated with propolis extracted from the hive of *T. calophyllae* displayed high expression of *GSC*. Further, the hPSCs treated with propolis collected from the nest of *T. travancorica* showed more expression of endoderm markers – *SOX17* and *SOX 7*. Propolis did not support the cells to differentiate into mesoderm or neuroectoderm lineage as there was no variation in expression of mesoderm marker (*MSGN1*) and neuroectoderm markers (*PAX6* and *NCAM1*). As per the findings, the propolis sample extracted from the nest of *Tetragonula* spp. supported the cells to differentiate into a mesendoderm lineage

The propolis extracts for the study was collected from live stingless beehives of three different bee species. Many studies have tested the effect of propolis on different cell lines *in vitro*; however, there are no reports on its effect on human induced pluripotent stem cells. Herein, we used *in vitro* experiments to study the cytotoxic effect of propolis and the gene expression of propolis treated cells. Our result revealed that propolis was not cytotoxic at low concentrations, increased the rate of cell proliferation; however, at higher concentrations they hindered cell growth. Cells treated with the propolis

extracted from *Lisotrigona* sp. (P1), *T. calophyllae* (P2) and *T. travancorica* (P3) obtained IC50 values of 410.904 neuroectoderm as there was no variation in the expression of neuroectoderm, 480.097 and 215.157 μg ml^{-1} respectively. These findings agreed with previously published studies, that identified that propolis could enhance the proliferation capacity of BMMSC (Elkheney et al., 2019) and stem cells derived from human exfoliated deciduous teeth (Fung *et al.*, 2015).

Propolis, a natural compound is known for its tissue regeneration activities. In the present study, when cells were treated with propolis, the cells lost their pluripotent state and started to differentiate. There was a rapid downregulation in the expression of pluripotency marker *NANOG* and *OCT4*. During embryonic development, the primitive streak initiates the differentiation of pluripotent epiblast cells into germ layers. That is, during differentiation, stem cells move into a transition state called primitive streak state/mesenendoderm state, Hence, transient primitive streak-like mesendodermal state is crucial for the differentiation of stem cells (Takahashi *et al.,* 2014). Goosecoid (GSC) is a mesendoderm marker (Jos *et al.*, 1998), cells when treated with propolis showed expression of *GSC*. Cells treated with propolis extracted from the nest of *T. calophyllae,* observed high expression of *GSC* compared to the other two propolis. This indicated that these cells displayed a high tendency to differentiate into meseoendoderm lineage. Endoderm lineage differentiation of cells treated with the propolis was determined by the expression of endoderm markers *SOX17*. However, propolis did not support the cells to differentiate into mesoderm and neuroectoderm as there was no variation in the expression of neuroectoderm markers (*PAX6 and NCAM1*) and mesoderm marker (Mesogenin 1(*MSGN1*)). The propolis extracted from the nest of *Tetragonula* spp. (*T. calophyllae and T. travancorica)* showed more tendency to differentiate into mesoderm and endoderm lineage compared to propolis extracted from *Lisotrigona* sp. Previous studies also reported that propolis enhanced the differentiation of stem cells. Elkhenany in 2019 reported that propolis increased the proliferation rate of bone marrow-derived mesenchymal stem cells (BMMSCs), enhanced the chondrogenic and adipogenic differentiation processes. Intraperitoneal injection of CAPE, a major component of propolis enhanced bone regeneration in the rat calvarial defect model (Ucan *et al.,* 2013) and studies also reported that ethanolic extract of propolis can promote bone regeneration and induce hard tissue bridge formation in pulpotom.

Stem cell therapy has revolutionized modern clinical therapy with the potential of stem cells to differentiate into different cell types which may help to replace different cell lines of an organism (Singh *et al.*, 2015). Natural compounds have been used in traditional medicine for the treatment of a wide range of diseases, further investigating their proliferative, differentiation, and cytotoxic effects on stem cells may provide a deeper understanding for curing various diseases. In the present study, propolis, a natural compound, supported the cells to differentiate into a particular lineage. Hence better understanding the chemical composition of propolis, investigating its mechanism and regulatory effects will pave the way as the invaluable candidates in future regenerative medicine research.

ACKNOWLEDGEMENTS

The Authors thank the Kerala Agricultural University. Authors are grateful to Dr. R. V Shaji for his support and the generous gift of the hiPSCs (D14C2). SM.S. acknowledges the financial support from the Indian Council of Medical Research (ICMR) (2019-3008/SCR/ADHOC/ BMS), and Council of Scientific and; Industrial Research (CSIR) (09/1108(13739)/2022-EMR-1), India.

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(Received July 31, 2022; revised ms accepted September 31, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.789
ENTOMON 47(4): 365-374 (2022)
Article No. ent. 47402

A new species of *Nesolynx* Ashmead, 1905 (Hymenoptera, Eulophidae) parasitizing potter wasp, *Delta pyriforme* (Fabricius, 1775) (Hymenoptera, Vespidae) in its nest from southern India

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ABSTRACT: *Nesolynx deltaphagus* **sp. nov.** parasitizing the potter wasp species *Delta pyriforme* (Fab.) (Hymenoptera, Vespidae) is newly described with illustrations from Kerala, India. This is the first report of parasitism of *Nesolynx* on Vespidae. A key for the Indian species of *Nesolynx* is provided along with the diagnosis of the new species with congeners. DNA barcode of the new species using universal primers of CO1 is also provided against accession number (Accession No: OK484482).

KEY WORDS: Chalcidoidea, Tetrastichinae, taxonomy, host record

INTRODUCTION

Nesolynx Ashmead (Eulophidae, Tetrastichinae) is a small genus having widespread distribution in the Neotropical and Oriental regions (Noyes 2019). The genus is presently represented by 17 described species worldwide, nine from the Oriental region and five species namely *N. flavipes* Ashmead, *N. javanica* (Ferrière), *N. orientalis* Khan, Agnihotri & Sushil, *N. phaeosoma* (Waterston) and *N. thymus* (Girault) are recorded from India (Bouèek, 1976, 1988; Narendran, 2007; Noyes, 2019). The majority of *Nesolynx* species are gregarious primary parasitoids on pupae of Hymenoptera (Braconidae and Ichneumonidae), Lepidoptera (Gracillariidae, Limacodidae, Notodontidae and Psychidae, Pyralidae) or Hemiptera (Pseudococcidae) pupae and also act as hyperparasitoids through Tachinidae (Ferrière, 1939; Bouèek, 1988; Narendran, 2007; Noyes, 2019).

The potter or mason wasp species *Delta pyrforme* (Fab.) (Vespidae, Eumeninae) are solitary wasps preying and provisioning their developing immature mainly with caterpillars in excellently sculpted earthen incubation chambers for their developing immature (Segoli *et al.*, 2020; Deshmukh, 2021). Even with the impregnable architectural finesse, the brood of the potter wasp are prone to attack. A strepsipteran parasite, *Stylops* sp. is found to attack *Eumenes petiolata* (=*D. pyriforme*) from India (Smith, 1859; Salt and Bequaert, 1929).

Members of eulophid genera *Elasmus* Westwood,

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Melittobia Westwood and *Kocourekia* Bouček are only known till date to attack aculeate Hymenoptera (Edwards and Pengelly, 1966; Krombein, 1967; Valentine, 1967; Bouček, 1977; Donovan, 1980; Dahms, 1984; Macfarlane *et al.*, 1984; Donovan and Macfarlane, 1984; Macfarlane and Palma, 1987; LaSalle, 1994; Okaba and Makino, 2008; Kim, *et al.*, 2016; Cao *et al.*, 2017; Noyes, 2019). A new species of *Nesolynx* attacking the pupa of *D. pyriforme* from southern India is described with illustrations.

MATERIALS AND METHODS

Nest making by a female *D. pyriforme* was observed during the early hours 9th December 2020 on a branch of *Phyllanthus acidus* (L.) from Elathur (11°19'32.3"N ; 75°44'30.3"E, alt. 23 m above mean sea level) in Kozhikode district of Kerala, India. The potter wasp moved to and forth bringing in lumps of semi-solid mud within its mandibles held on by forelegs and carefully moulding the nest using its mandibles at an average of 12±3 seconds per lump. A single egg was placed after mass provisioning each cell at an average of 9±3 larvae per cell. Individual cells were closed with a narrow seal (through which the emerging wasps chew their way out). The entire nest with four chambers (three adjoining the branch and one exterior) was finished (6.5×3.5×2.5 cm) by late 14th December 2020. The nest was broken off from the branch after observing exit holes visible outside on 13th January 2021. On detaching, an intact pupal film was observed in one of the cells (Figs. 19, 20). Adult parasitoids emerged out of the film immediately and the nest along with emerging parasitoids were quickly transferred into a clear container. The adult potter wasp was identified by Dr. Girish Kumar (Vespidae expert, Zoological Survey of India, Western Ghat Regional Centre, Kozhikode, Kerala).

Parasitoids were carefully aspirated, killed and stored in 70 percent ethyl alcohol, processed using standard Hexamethyldisiloxane treatment (Heraty and Hawks, 1998). Morphoanalysis of the specimens were done under Leica M205A Stereo zoom Microscope and images were captured using Leica DMC 2900 digital camera attached to the microscope. Measurements of the specimens were obtained using Leica LAS (Leica Application Suite V4.7.1) microsystems by Leica (Heerburg, Switzerland). Images at varying focal planes were stacked into a single image using Leica Automontage Software V4.2 and final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® CS5 (Version 12.0 x64). Molecular analysis was carried out using NucleoSpin® Tissue Kit (Macherey-Nagel) (DNA isolation) and PCR amplification of a 591 bp region near the 5' terminus of the CO1 gene following standard protocols and universal primers (Folmer *et al.*, 1994). The amplified sequence was analysed using Geneious Pro v5.1 (Drummond *et al.* 2010), compared in online BLAST and uploaded to NCBI (Accession No: OK484482). The new species was also compared with the holotype images of *N. flavipes* Ashmead [USNMENT00802939] available in the entomological collection of National Museum of Natural History, Washington, U.S.A (NMNH, previously USNM).

The description of the new species is based on the type specimens deposited in the "National Zoological collections" of Zoological Survey of India, Western Ghats Regional Centre, Kozhikode (ZSIK).

Terms and measurements. The terms used are mainly those of Narendran (2007) unless noted otherwise. The nomenclature for cuticular sculpture follows Harris (1979). Abbreviations of terms used are as follows: AOL = distance between anterior ocellus and posterior ocellus; CC = costal cell; fu_x = funicular number; Gt_x = gastral tergum number; ML = median line or groove; MS = malar space; MV= marginal vein; OOL = oculo-ocellar distance, minimum distance between a posterior ocellus and eye; POL = postocellar distance, the distance between the two posterior ocelli; SMG = submedian groove; SMV = submarginal vein; STV= stigmal vein; UNC = uncus.

RESULTS AND DISCUSSION

Genus *Nesolynx* Ashmead, 1905

Nesolynx Ashmead 1905. 28: 966. Type species: *Nesolynx flavipes* Ashmead, by monotypy.

Ceratotrastichus Girault and Dodd in Girault 1913: 254. Type species: *Ceratotrastichus bisulcatus* (synonymy by Bouček, 1988).

Omphalomomyia Girault 1913: 174. Type species: *Omphalomomyia lividicaput* Girault (synonymy by Bouček, 1988).

Aceratoneurella Girault 1917: 7. Type species: *Aceratoneurella cinctiventris* Girault, (synonymy by Bouček, 1977).

Diagnosis. Head with vertex not collapsing; antenna short with funicular segments usually transverse; mesoscutum without ML; mid lobe of mesoscutum with dense and regular pilosity, each hair placed on small papillae; scutellum without SMG, anterior setae of scutellum well before middle; petiole very short, hardly visible.

Note. A key to Indian species is augmented from Narendran (2007) and modified to incorporate the new species, *N. deltaphagus*. Narendran (2007) states *N. orientalis* as a junior synonym of *N. javanica*, but this could not be validated in the present study due to lack of additional specimens and unavailability of type specimens on request. Hence, we retain the nominal status of the taxa and include the same in the Indian key.

Key to the Indian species of *Nesolynx* Ashmead

1. Mesosoma orange; metasoma orange, with dorsal margin black; vertex and frons black with metallic green reflections, lower face yellowish brown.....***thymus* Girault**

- Mesosoma black or dark brown with or without metallic tinge; metasoma mostly black or brown; vertex and frons black without metallic green reflections, lower face black or dark brown **2**

2. Mid lobe of mesoscutum with long semierect pilosity becoming larger posteriorly; wing without dense discal pubescence; flagellum pale yellow, brown on apical flagellomeres; metasoma longer than combined length of head and mesosoma; hind basitarsus shorter than second tarsomere ..... ***phaeosoma* (Waterston)**

- Mid lobe of mesoscutum with short and uniform pilosity; wing with dense discal pubescence; flagellum dark brown; metasoma as long as or little shorter than combined length of head and mesosoma; hind basitarsus longer than second tarsomere..... **3**

3. Gt_1 longest, at least reaching one-third of gaster, following tergites subequal; pedicel less than 2.0× as long as broad; MV 4–6× as long as STV; body shiny black or brown with slight metallic reflections on mesoscutum and metasoma; all legs yellow including forecoxa..... **4**

- All gastral segments almost equal; pedicel greater than 2× as long as broad; MV up to 4× as long as STV; body black without metallic reflection; all legs yellow with at least fore coxa black..... **5**

4. Frontovertex wide, 0.7× of total head width; mouth more than 2× broader than MS; POL 1.2× OOL; OOL greater than AOL; fore wing with decolourised area on parastigma..... ***deltaphagus* sp. nov.**

- Frontovertex 0.5× total head width; mouth slightly broader than MS; POL 3× OOL; OOL less than AOL; fore wing without decolourised area on parastigma..... ***flavipes* (Ashmead)**

5. Antennae situated at the level of ventral margin of eyes; fu_2 slightly longer than broad and fu_3 subquadrate; clava shorter than the combined length of two preceding funicular segments; scutellum with two pairs of setae; SMV with four setae; MV as long as SMV.....***javanica* (Ferriere)**

- Antennae situated well above the level of the ventral eye margin; fu_2 quadrate and fu_3 wider than long; clava longer than the combined length of two preceding funicular segments; scutellum with three pairs of setae; SMV with five setae; MV shorter than SMV..... ***orientalis* Khan, Agnihotri & Sushil**

***Nesolynx deltaphagus* sp. nov.** (Figs. 1–16)

LSIDurn:lsid:zoobank.org:act:2740AF9F-B79A-42FA-B4F8-FE8F1D2CB661

Type material: Holotype: ♀ India: Kerala, Kozhikode district, Elathur (11°20'37"N;

75°43¹6.74ºE, alt. 23m above mean sea level), 13.i.2021, Coll. C. Binoy, ex. pupa of *Delta pyriforme* (Fab.). Paratypes: 293♀, 35♂, same data as the holotype.

Depositories: Holotype ♀ [ZSIK] ZSIK Regd. No.ZSI/WGRC/IR/INV.21914, Paratype ♀

[ZSIK] ZSIK Regd. No. ZSI/WGRC/IR/INV.21915

Diagnosis: Body brownish black with metallic reflection on head and metasoma, all legs yellow with infuscations on fore and hind coxae, frontovertex wide, 0.7× total head width, POL 1.2× OOL, apical margin of clypeus bilobed, emarginated with deep median cleft; mandible with strong tooth and truncation, fore wing with dense discal setation, decolourised on parastigma (between SMV and MV), propodeum reticulated with distinct median carina, metasoma ovate, slightly shorter than combined length of head and mesosoma, Gt_1 longest, 0.3× as long as metasoma, smooth and shiny on anterior half, remaining terga reticulate dorsally.

Description: Holotype ♀ (Figs. 1–11) Body length 0.90 mm, length of fore wing 0.71 mm.

Body brownish black with metallic reflections on head, mesosoma and metasoma. The following parts variably coloured: eye and ocellus reddish brown; scape and pedicel pale yellow, rest of antennomeres dark brown; all legs yellow except fore coxa and base of hind coxa (yellowish brown), metatibial spur pale yellow, claws brown; frons and vertex dark metallic green; supraclypeal area yellowish brown, clypeus yellow with apical margin reddish brown; mandible yellow with ventral margin and apex reddish brown; maxillary palpi pale yellow; pronotum brown with slight metallic greenish lustre; mesoscutum and scutellum dark shiny brown; mesepimeron and mesepisternum brown, acropleuron and tegula pale yellow; metasoma brown with coppery lustre; all terga with slight metallic reflection on apical margin; wings hyaline with veins and setae pale brown (Figs. 1–11).

Head in dorsal view transverse, 2.6× as broad as long, vertex shiny metallic, finely reticulate with scattered setigerous pits; ocelli arranged in about obtuse angled triangle; POL 1.2× OOL, OOL 1.7× AOL, POL 2.1× AOL (Fig. 6); in frontal view head 1.2× as wide as its maximum length, sculpture same as that of vertex, setae arising from each pit; toruli inserted at ventral eye margin (Fig. 3); mouth 2.5× broader than malar space; clypeus bilobed, strongly emarginated, with a deep cleft medially; mandible bidentate with strong tooth and truncation (Figs. 3–5); eye pubescent, height of eye in profile 2× as long as malar space; malar sulcus distinct, curved at base; malar space finely reticulate, no setigerous pits (Fig. 4); antenna with two distinct annelli, three-segmented funicle and three segmented clava (11233); scape and pedicel with short adpressed setae; long sensillae and numerous adpressed setae on remainder of antennomeres; scape not reaching median ocellus, reaching only 0.7× of frons, 3.2× as long as wide, 2.1× as long as pedicel; pedicel 1.8× as long as wide, 1.7× as long as fu_1; all funicles subquadrate; fu_3 1.2× longer than fu_1, 1.1× longer than fu_2; clava as long as combined length of all funiculars, 2.6× as long as wide, tapering to apex, distinct terminal spine present; relative length: width of antennomeres: scape = 75:23.5, pedicel = 41:22, fu_1 = 23.8:24.7, fu_2 = 24.8:26.5, fu_3 = 27.7:30.3, clava = 80:31 (Fig. 2).

Mesosoma: Pronotum and mesoscutum distinctly pilose with setae arising from well-arranged rows of pits; surface transversely reticulate similar head; pronotum subconical with raised projecting spiracle on either side and five pairs of long setae near apical margin; mesoscutum 1.5× as long as wide with well-marked notauli and a pair of longer setae near posterior margin of mesoscutum; posterior part of lateral lobe of mesoscutum bare; axilla with similar sculpture as mesoscutum (Fig. 7); scutellum longitudinally reticulated with distinct sublateral grooves, 1.4× wider than long, having two pairs of long suberect setae, anterior setae placed towards anterior margin of scutellum; axillula reticulated without conspicuous setae; propodeum and dorsellum with wider reticulations; dorsellum 4× as long as wide, half-length of propodeum; propodeum short, 4× as long as broad, median carina distinct (Fig. 9); propodeal spiracle large, peritreme exposed, nearest to anterior margin, distance from anterior

Fig. 1-11 *Nesolynx deltaphagus* **sp. nov.** Holotype ♀. 1, habitus, lateral view; 2, antenna; 3, lower face (clypeus), frontal view; 4, head, lateral view; 5, head, frontal view; 6, head, dorsal view; 7, mesosoma, dorsal view, 8, mesosoma, lateral view, 9, propodeum, dorsal view, 10, fore wing, 11, metasoma, dorsal

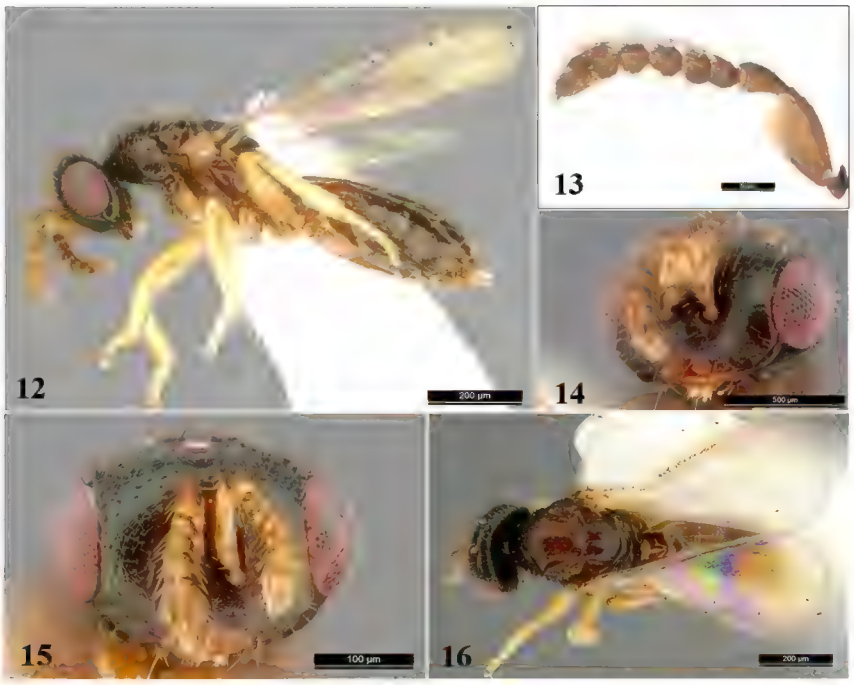

Fig. 12-16 *Nesolynx deltaphagus* **sp. nov.** Paratype ♂. 12, habitus, lateral view; 13, antenna; 14, lower face (clypeus), frontal view; 15, head, frontal view; 16, habitus, dorsal view

Fig. 17-20 *Delta pyriforme* (Fab.) Nesting and parasitisation. 17, ♀. *D. pyriforme* building nest on *Phyllanthus acidus* (L.) (December, 2019); 18, finished nest; 19, *N. deltaphagus* emerging out of pupal film of *D. pyriforme*; 20, emerged nest of *D. pyriforme* (arrow indicating the cell harbouring parasitised *D. pyriforme* pupa)

margin to spiracle less than diameter of spiracle; callus with three setae; lateral panel of pronotum and prepectus reticulate; acropleuron smooth; mesepisternum almost smooth, transepisternal sulcus present; upper mesepimeron and lower mesepimeron smooth and shiny, transepimeral sulcus almost straight; metapleuron weakly reticulate (Fig. 8).

Wings: Fore wing hyaline, with short and dense discal setation, 1.9× as long as its maximum width; SMV with three strong semi erect dorsal setae; decolourised area on parastigma between SMV and MV; STV terminates in small rounded knob; cubital setal line wavy up to middle, remainder straight till apical wing margin; MV 1.8× as long as SMV; MV 5.3× as long as STV; UNC relatively long and slender, half-length of STV; costal cell 6.6× as long as broad, with single line of setae; basal cell having four small setae; basal vein with three setae, speculum closed below, marginal fringe on wing short; subcubital line nearest to the posterior wing margin; hind wing hyaline, 4.8× as long as wide, marginal fringe long, discal ciliation similar to that of fore wing (Fig. 10).

Legs: Metacoxa setose, weakly reticulate, 2.4× as long as wide; hind femur pubescent, medially widened and tapering at both ends, surface reticulate, 2.4× as long maximum width; hind tibia densely setose, 1.1× as long as hind femur, 6.2× as long as wide; metatibial spur short, not reaching middle of basitarsus (Fig. 1).

Metasoma: Petiole hardly visible in dorsal view; metasoma ovate, basal one third of Gt_1 smooth and shiny, remaining part reticulated with metallic reflections; densely setose, 1.2× longer than mesosoma, 0.1× shorter than combined length of head and mesosoma, 1.54× as long as broad (Fig.1); Gt_1 relatively large, posterior margin slightly emarginate, 0.3× as long as metasoma, Gt_2–Gt_4 subequal in length; Gt_5 1.3× longer than preceding tergum; Gt_6–Gt_8 short, without metallic reflections; hypopygium reaching middle of metasoma; ovipositor slightly protruding; cercal setae unequal, longer one sinuate (Fig. 11).

Male Description: Paratype ♂ (Figs. 12–16). Body length 0.98 mm, length of forewing 0.63 mm. Surface sculpture similar to that of female. The following characters (other than usual sexual dimorphic states on number of antennomeres, size and terminal metasomal segments) may be considered in associating the male of *N. deltaphagus* **sp. nov.** from its female. Hind femur infuscated medially (Fig. 12); scape apically expanded into a plaque, 2.0× as long as maximum width; pedicel 2.2× fu_1; each funicle bearing a whorl of long bristle like setae, setae 2.5× longer than respective funicular length (Fig. 13); eye conspicuously setose (Figs 14, 15); metasoma 1.1× as long as the combined length of head and mesosoma (Figs. 12, 16).

Distribution: India: Kerala.

Host: Gregarious parasitoid on pupa of *Delta pyriforme* (Fabricius, 1775) (Figs. 17–20).

Etymology: The species epithet is derived from the host's genus name *Delta.*

Remarks: The new species resembles the Oriental species *N. javanica* (Ferrière) in the key to Oriental species of *Nesolynx* (Narendran, 2007) in having propodeum with median carina, fore wing with dense discal setation, but differs from the same in having OOL greater than AOL (*vs.* OOL less than AOL), POL 1.2× OOL (*vs.* POL 3× OOL), club as long as combined length of all funiculars (*vs.* club not longer than combined length of fu_2 and fu_3); 5.3× as long as STV (*vs.* MV 4.0× STV), metasoma 0.9× as long as combined length of head and mesosoma (*vs.* metasoma as long as combined length of head and mesosoma), Gt_1 longest (*vs.* gastral terga subequal in length), metasoma brown with slight metallic reflection (*vs.* metasoma without any metallic reflection).

N. deltaphagus **sp. nov.** is similar to *N. flavipes* Ashmead in having body black or brown with slight metallic on metasoma; Gt_1 longest and median carina on propodeum distinct. However it differs from *N. flavipes* in having: fore and hind coxae infuscate (*vs.* all legs yellow); fore wing with veins brown (*vs.,* fore wing with veins yellow); POL 1.2× OOL (*vs.* POL 3.5× OOL); OOL greater than AOL

(*vs.* OOL less than AOL); mouth 2.5× broader than MS (*vs.* mouth 1.2× broader than MS); mesoscutum coarsely reticulate with small setigerous pits (*vs.* mesosoma with large setigerous punctures); metasoma 0.9× as long as combined length of head and mesosoma (*vs.* metasoma as long as combined length of head and mesosoma).

Nesolynx is a group of gregarious parasitoids on pupae of various holometabolous insects and a few species are known to possess various desirable attributes of a biocontrol agent (Kumar *et al.*, 1996; Narayanaswamy and Devaiah, 1998; Aruna and Manjunath, 2009). The potter wasp nest is mostly impenetrable to any foreign entity when it is completely moulded for incubation. The only report of a parasite on *D. pyriforme* is recorded by Smith (1859: 130) — *Eumenes petiolata,* ♀, India. The abdomen of the third segment with a female *Stylops* beneath it, at the fourth distorted by the pupa case of an escaped male — and subsequent confirmation by Salt and Bequaert (1929: 253). The present study forms the first report of any parasitic hymenopteran attacking the potter wasp *D. pyriforme.*

Molecular analysis and need for a deeper probe

Neighbour Joining trees (NJ trees) are quick visual summaries of degrees of specialization of a species, indications of taxonomic puzzles, variability in barcode length, BIN composition (the equivalent of sorting morphological look-alikes into unit trays), and data-checking and typically conspecifics grouped together in their own terminal clade (Sharkey *et al.*, 2021). However, specimens with less than about 550 base pairs are more likely to be misplaced on the tree, often that requires other traits (morphology and/or host data in case of parasitoids). This leads to the placement of unrelated specimens to group together (lack of conserved base pairs). BLAST inventory of successfully barcoded conspecifics (irrespective of nomenclature) should also be present which is the primary requisite for any successful NJ tree preparation and if in absence, the resolution of such species complexes requires ecological, morphological, and a deeper genomic probe (Janzen *et al.*, 2017 and references therein).

The CO1 genomic extracts of *N. deltaphagus* **sp. nov.** subjected to PCR amplification yielded a 591 bp (forward) and 377 bp (reverse) region near the 5' terminus standard protocols. The forward and reverse sequences were analysed using the online BLAST tool of NCBI for comparison with congeners. Because of the absence of any other related taxa in the database, the new taxa formed an outgroup to all deposited eulophid material and could not be represented on an NJ tree. Henceforth, this deposited sequence could be used in future reference of the *Nesolynx* taxa.

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of Malabar Christian College, Calicut and the Department of Zoology, the University of Calicut for providing necessary infrastructure and facilities. Thanks are due to Dr. Girish Kumar Scientist-C, ZSI WGRC, Kozhikode for the identification of the potter wasp species. The authors thank the authorities at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala (RGCB) for their help rendered in the molecular analysis of the new species. JRV and CB thankfully acknowledge UGC for the financial support by means of UGC JRF and NFSC respectively. SS acknowledges the financial assistance from SERB, Department of Science and Technology, Govt of India, New Delhi, for the financial assistance (File No. EMR/2017/005528).

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(Received July 04, 2022; revised ms accepted October 10, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.790
ENTOMON 47(4): 375-382 (2022)
Article No. ent. 47403

Forensic implications of the seasonal changes in the rate of development of the blowfly, *Chrysomya megacephala* (Fabricius) (Diptera, Calliphoridae)

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ABSTRACT: Studies on the development rate of *Chrysomya megacephala* (Fabricius) suggested that the blowfly as a significant candidate for forensic investigations. Under natural ambient conditions development rate of *C. megacephala* in monsoon, winter and summer seasons indicated significant differences among seasons. The larvae began pupation at 92nd h in summer, 157th h in the monsoon season and 191st h in winter. Rapid larval growth in terms of length was observed in summer. During summer, the length of the larvae increased to a maximum of 13.9 mm at 54th h. Time taken for the emergence of the adult fly was 164, 249 and 311h in summer, monsoon and winter seasons respectively. Life table studies were conducted to assess the percentage survival and mortality by recording the survival rate of different development stages. Molecular diagnosis of species was done using COI gene. The analysis included molecular sequences of other samples of the same species from different regions of India. The neighbor-joining method allowed us to identify the species at molecular level with precision and accuracy.

KEYWORDS: Larval growth, pupation, adult emergence, seasonal differences, life table, molecular diagnosis

INTRODUCTION

The blowfly, *Chrysomya megacephala* (Fabricius) (Diptera, Calliphoridae), a synanthropic fly, commonly known as oriental latrine fly inhabiting human settlements and commonly seen on decomposing cadavers, fish, carrion, human feces and sweet materials; indicating its medical, veterinary and forensic significance. Due to their cosmopolitan distribution, ubiquity and abundance, *C. megacephala* is recognized as the one of the most important species of insects in forensic entomology (Badenhorst and Villet, 2018). The larvae feed and grow on soft tissues of living and dead vertebrates especially mammals, birds and fish (Yang and Shiao, 2012). The adult flies were usually attracted to decaying cadavers and reach within a few hours of death of the animal (Zumpt, 1965), and it has been considered as an important fly for the determination of minimum postmortem interval (Wang *et al.*, 2008). Medico legal cases world over have reported the forensic relevance of *C. megacephala* (Gruner *et al.*, 2017; Richards and Villet, 2009; Amendt *et al.*, 2004; Goff and Flynn, 1991). For the determination of minimum postmortem intervals, age of larvae will be helpful (Gruner *et al.*, 2017). Studies focusing the

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development of *C. megacepehala* has been done previously (Subramanian and Mohan, 1980; Bharti *et al.,* 2007; Sukontason *et al.,* 2008; Niederegger *et al.,* 2010; Bala and Singh, 2015; Zhang *et al.,* 2018).

Age grading studies on immature stages of *C. megacephala* at different temperatures in the laboratory has been done at Punjab, India where the fly took 6.3 days for development from egg to adult stage at 30°C (Bharti *et al.*, 2007; Bala and Singh, 2015). Estimates of postmortem interval (PMI) based on the known characteristics of the infesting fauna in the natural conditions of the specific geographical location are very important (Sukontason *et al.*, 2008). Niederegger *et al.* (2010) suggested that negligence of fluctuating temperatures in legal cases can lead to distinctly wrong estimates of the PMI. The studies targeted on the time since death assessment has been recognized to be scanty in the present scenario. Studies were undertaken to reveal the seasonal changes in the developmental rate of *C. megacephala* during summer, monsoon and winter season to develop an accurate estimation of PMI.

MATERIALS AND METHODS

Rearing of *C. megacephala:* The adult flies were reared in the outdoor open system rearing facility in Kolangattukara, Choolissery, Thrissur district, Kerala, India (10° 352 34.873 N; 76° 112 22.63 E) during summer, monsoon and winter season. Adult females of *C. megacephala* were trapped and isolated in the rearing cabinet with decomposing pork meat as bait. The insects were reared in triplicate in the rearing cabinets (60 cm × 30 cm ×30 cm). Relative humidity, rainfall and temperature were monitored in May, July, August and December (2019) and January (2020). The average temperature and relative humidity in the respective months were 31.15 ±2.26°C, 27.71± 1.47°C, 26.09±1.35°C and 72.30±10.84, 88.07±4.21, 71.46±17.73 per cent respectively. The average rain fall recorded during July-August months was 1999.7mm. The adult insects were provided with 10 per cent (w/v) sugar solution and 1.5 per cent (v/v) multivitamin syrup solution and water as food and liquid sources (Byrd, 2001; Von Aesch *et al.,* 2003). The decomposing pork meat served as a reflex stimulus for the adult female fly to lay eggs and also served as a food source for the larvae. Wet vermiculite was kept as the bottom layer in the cabinet to assist the migration of third instars for pupation. Few of the blowflies trapped were killed and pinned as dry specimens for morphological identification and few were preserved in ethanol (70%) for molecular identification. The morphological examination was done with LEICA-S8APO stereomicroscope. Six numbers each of eggs, different larval instars and pupae were randomly collected every six hours for further studies.

Observations were done regularly on an hourly basis to detect the presence of eggs. Once the eggs were found, the eggs with the bait were transferred to the larval rearing plastic jars. Wet vermiculite was laid at the bottom of the jar to maintain adequate humidity. The jar was covered with a wet cotton cloth to prevent the entry of other insect parasitoids. Fresh pork meat 50 g was put in to the jar as larval feed. This was continued until the instars reached the non-feeding stage and started pupation. Fresh pupae were collected and transferred to a new rearing jar with moist vermiculite at the bottom and it was kept inside the rearing cabinet for the emergence of the adult fly. Different larval instars were collected for studying their morphology and length parameters. The adult flies were identified using morphological keys provided in the standard literature (Senior-White *et al.,* 1940; Bharti, 2019).

Life table study: Life table studies were conducted to assess the percentage survival and mortality by recording the survival rate of different development stages. Survival studies were undertaken in all seasons in triplicate. In each replicate trials of rearing, survival rate in percentage was calculated for each stage of the life history; egg, 1st, 2nd, 3rd instars, post feeding stage and pupa till the emergence of adult flies. Average number of eggs considered for rearing in each replicate of the triplicate trials in summer, monsoon and winter seasons were 124,121 and 128 respectively. The time of oviposition till the emergence of adult fly

was considered for the study. The time taken for egg hatching was noted. The freshly hatched larvae were transferred to the new larval rearing chamber and 50 g of fresh pork meat was provided as food. Ten larvae were collected every six h and boiled for two minutes at 96-99°C and preserved in alcohol (70 %) for the assessment of length (Adams and Hall, 2003). The time spent in each life stage was recorded. Based on these observations growth curves were plotted. The effect of temperature, relative humidity and rainfall on larval development was also studied.

Molecular characterization was done using Cytochrome oxidase Subunit I (COI) gene. The isolated sequence was submitted in GenBank, NCBI with Accession No: MW 522614. The data were statistically analysed using SPSS 24.0.0. The relation between the temperature and various life stages from 1st, 2nd, 3rd instars and post feeding stages was analysed in one-way analysis of variance.

RESULTS AND DISCUSSION

The adults emerged were identified as of *C. megacephala*. The growth curve was sigmoid during the summer, monsoon and winter seasons (Fig. 1). The time taken for the emergence of the adult fly was long during the winter season (December-January) at an average atmospheric temperature of 26°C (F = 475.7 at df = 28). The maximum length of larvae reached at 138th hour during winter season (F = 120.2 at df = 8). The time taken for the emergence of the adult fly was shorter in the summer season (May) at an average temperature of 31°C (F = 837.0 at df = 14). During summer maximum length of larvae reached at 54th h of development (F = 179.8 at df = 2). The time taken for the development during the monsoon (August-September) (F = 591.9 at df = 23) was found at 28°C, which was slightly longer than that of the summer season. During monsoon season

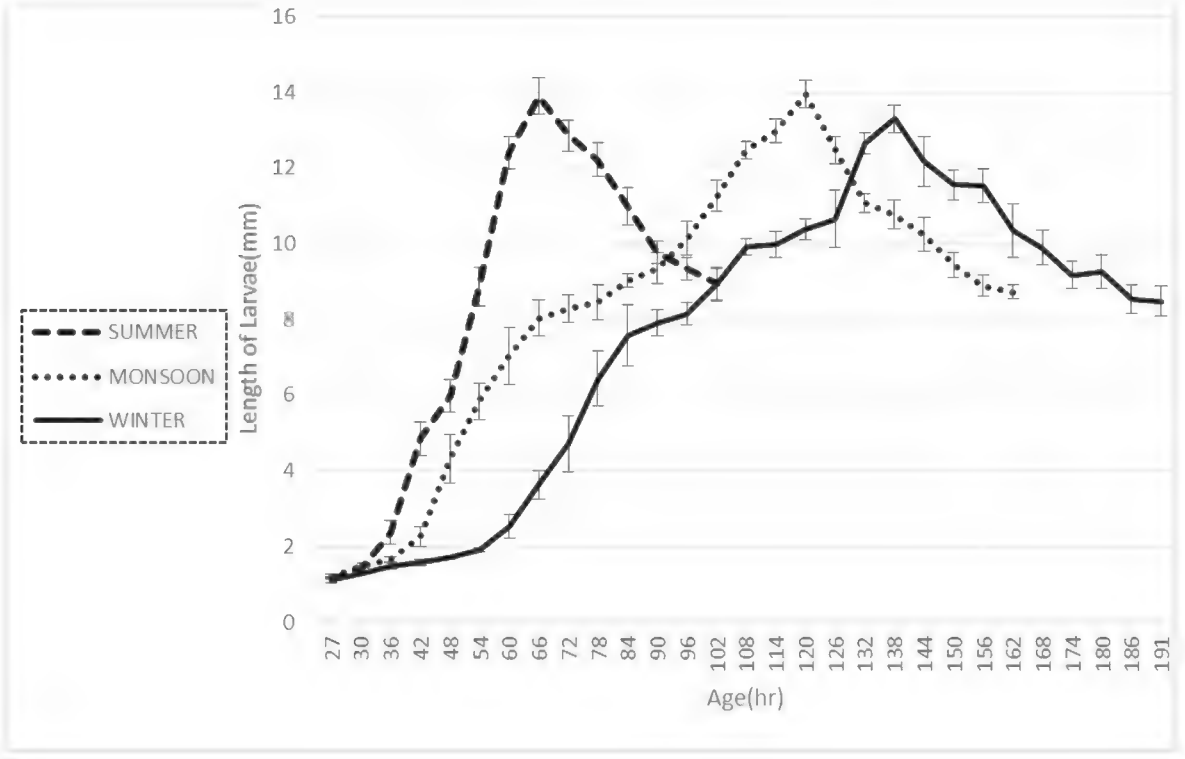
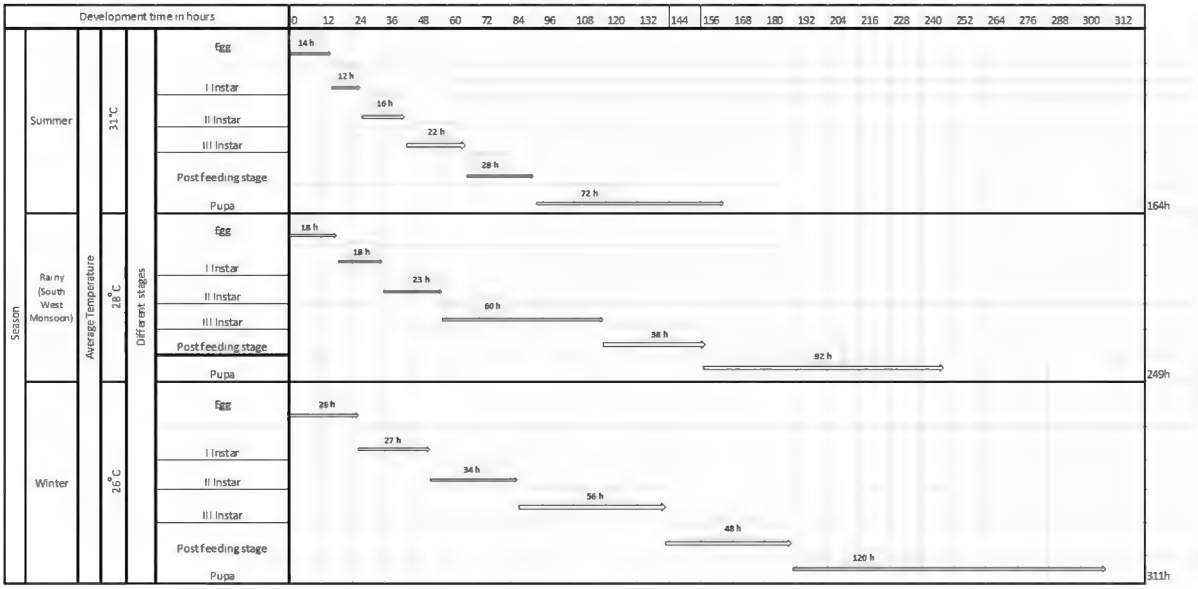


Fig. 1 Developmental rate of *Chrysomya megacephala* from newly hatched larvae until pupation under natural conditions in Kerala, India in Summer, Monsoon and Winter Seasons (black bars indicate mean ± SD)

Fig. 2 Evolutionary analysis of *SR1859-COI-F_E03 Chrysomya megacephala* by Maximum Likelihood method

Table 1. Life table showing rate of development of different stages of *C. megacephala* with respect to age in different seasons in Kerala, India (mean value of all replicates)



maximum length of larvae reached at 114th hour (F = 203.715 at df = 9).

As illustrated in the life table, the pupation started at 191st h in winter season, 157th h in monsoon season and 92nd h in summer season. Life table showing rate of development of larval stages of *C. megacephala* with respect to age after egg hatching till commencement of pupation during different seasons is presented. During summer, the development of the larvae was faster with rapid increase in body length. After attaining the maximum size, when the post-feeding stage started, the length of the larvae reduced (Table 1).

Molecular diagnosis of species was done using COI gene. The isolated sequence was submitted in GenBank, NCBI with Accession No: MW522614. It displayed 99.84 per cent identity with sequence of same species collected from China (Accession No. MK075772.1). *C. megacephala* has also been identified using barcoding in northern and southern part of India, with Accession No: AB910390 (Ramraj *et al.*, 2014), KX893351, KX893341, KX893343, KX893342, KX893346, KX893347, KX893344, KX893345 (Bharti and Singh, 2017). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The neighbour-joining method allowed us to identify the species at molecular level with precision and accuracy. The optimal tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree (Fig. 2). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. This analysis involved 28 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 638 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.*, 2021)

Life table studies provided the survival rate and mortality rate of each development stage. Survival studies were undertaken in all seasons in triplicate. Maximum hatching was observed during monsoon season (92.18%) and lowest during winter season (85.12%). Similarly 92.18 per cent of first instar larvae became second instar during monsoon season closely followed by summer (89.51%) and the lowest in winter (71.07%). The same trend was followed in the case of third instar larvae with 74.19, 80.46 and 61.15 per cent respectively for summer, monsoon and winter seasons; and reached pupal stage with 61.29, 71.09 and 51.23 per cent respectively. A total of 68, 84 and 47 adult flies emerged from pupae respectively for summer, monsoon and winter seasons. Total survival rate for *C. megacephala* during summer, monsoon and winter was found to be 54.83, 65.62 and 38.84 per cent respectively. The total time taken for the development of was found to be 164, 249 and 311h in summer, monsoon and winter respectively (Table 2).

In forensic investigations, apart from the species identification of the blow fly, the knowledge of the rate of development of the blow fly in the specific geographical location is very crucial in the accurate estimation of Post mortem Interval (PMI). The metabolic rate of the insects increase with the increase in temperature (Anderson, 2000). Byrd and Butler (1997) reported that the results of developmental rate of *C. ruffifacies* conducted at constant temperature could be applied to fluctuating temperature conditions. The developmental rate of *C. dubia* at fluctuating temperatures were similar to that conducted at a mean constant temperature (Dadour *et al.*, 2001). In present study the time taken for pupation during monsoon season (27°C) was 139h which is close to the results (144h at 27°C) obtained on the same species by Wells and Kurahashi (1994) and 132h at 28°C by Sukontason *et al.* (2008). Wells and Kurahashi (1994) also reported that the total time taken for adult emergence was 234h; which is similar to the total time of 249h recorded in the present investigation. The seasonal study on development rate conducted on *C. megacephala* in the present study showed with a shortest period of onset of pupation from newly hatched larvae at summer followed by monsoon season and then the winter. This results are reaffirming the observations made by Smith (1986) in which the higher temperature increased the larval

Table 2. Survival rate of different life stages of *C. megacephala*

Stage	No. of each stage			Survival rate at each stage (%)			mortality rate at each stage (%)		
	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter
Egg	124	128	121	94.35	95.71	85.12	5.65	4.29	14.88
1st Instar	117	122	103	89.51	92.18	71.07	10.49	7.82	28.93
2nd Instar	111	118	86	74.19	80.46	61.15	25.81	19.54	38.85
3rd Instar	92	103	74	61.29	71.09	51.23	38.71	28.91	48.77
Pupa	76	91	62	54.83	65.62	38.84	45.17	34.38	61.16
Adult fly	68	84	47	-	-	-	-	-	-

activity of these fly larvae, while the cold weather was inhibiting the fly activity. Subramanian and Mohan (1980) reported that at 25.5°C pupation time for *C. megacephala* was 150h in comparison to 165h at 26°C in the present study. The results of the present study are in line with studies of Goodbrod and Goff (1990) and Wang *et al.* (2018). While development rate obtained during monsoon season in the present study for *C. megacephala* are rather different from (Bharti *et al.*, 2007), but development rate conducted during summer season and winter season was rather similar to their study. In the present study, the time from hatching till pupation was 78h ,139h and 165h at summer, monsoon and winter season at an average temperature of 31°C, 28°C and 26°C respectively, while in the study of Bharti *et al.* (2007), the time taken was 69h, 94h and163h at 30°C, 28°C and 25°C respectively. This might be due to the changes in humidity, rainfall and temperature prevailing in these geographically different areas. Differences in developmental rate under constant temperatures are probably due to genetic variations of common flies with a worldwide distribution (Tourle *et al.*, 2009). The changes in the developmental rate of species during different seasons cautions that while performing the assessment of PMI, the investigators should be very careful about the climatic conditions prevailing in the respective study area (Gallagher *et al.*, 2010). This signifies the importance of generating location specific data of forensically important species for accurate assessment of postmortem interval. This is the first report on the developmental rate of this species during different seasons from South India and useful for the PMI assessment of dead bodies under forensic investigations during different seasons in future.

ACKNOWLEDGMENTS

The authors sincerely thank the Regional Facility for DNA Fingerprinting (RFDF), Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala for the DNA sequencing work. The authors also thank the Principal and Management of St. Thomas' College (Autonomous), Thrissur, Kerala, India for providing facility and support for conducting the present research work.

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(Received June 29, 2022; revised ms accepted November 01, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.791
ENTOMON 47(4): 383-390 (2022)
Article No. ent. 47404

Susceptibility of *Aedes albopictus* (Skuse, 1894) against the organophosphorus insecticide temephos, in Chidambaram, Tamil Nadu, India

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ABSTRACT: Investigation showed that *Aedes albopictus* (Skuse, 1894) of Chidambaram-Town, Annamalai-Nagar and Muthiah-Nagar of Tamil Nadu are still susceptible to the insecticide organophosphorus temephos with 98–100 mortality percentages. The resistance ratios of all the three sentinel sites are negligible. LC_{50} value was 0.002 - 0.003 ppm with high significance. It was the first temephos bioassay case study conducted on DENV vector *Ae. albopictus* in the selected sentinel sites and estimated lethal concentrations.© 2022 Association for Advancement of Entomology

KEY WORDS: Dengue, vector, mortality, resistance ratio, sentinel site

INTRODUCTION

World Health Organization dengue reported, increase of 8-fold cases over the last twenty years (Park *et al.,* 2022). The primary vectors for dengue virus are mosquito species belonging to the genus *Aedes* and *Ae. albopictus* (Skuse, 1894) plays a crucial role in the transmission of dengue virus–DENV (Rebecca, 1987; Muthusamy *et al.,* 2015; Amorin and Birbrair, 2022; Dalpadado *et al.*, 2022; WHO, 2022). Dengue track record in India is engrossing. It first debuted in 1780 (Chaturvedi and Nagar, 2008) and then reappeared in 1963–64 in East-Coast India (Pavri *et al.*, 1964; Chatterjee *et al.*, 1965; Carry *et al.*, 1966). Thereafter, frequent cases are reported from different parts of India against all four dengue virus (DENV) serotypes (Dash *et al.*, 2004; Dar *et al.*, 2006). Currently, dengue is prevalent throughout the country and in Tamil Nadu in all the districts since 2000 (Samuel *et al.*, 2021). Chemical control measures have been employed heavily to keep the vector population in check (Horstick *et al.*, 2010). In such scenario, application of temephos has gained momentum for elimination of immature *Aedes* mosquitoes in many countries (Ponlawat *et al.*, 2005; Jacquet *et al.*, 2015) as well as in India (Mukhopadhyay *et al.*, 2006). It is a non-systemic organophosphorus insecticide, used to control mosquito larvae and other insect pests. It was initially registered by US EPA in 1965 (by American Cyanamid Co, now BASF) and re-registered in 1991, and in India temephos is registered as 50 per cent EC for dengue mosquito larvae control (WHO, 2011). However, prolonged application of such measure has led to detection of insecticide

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resistance in a vector (Ocampo *et al.*, 2011; Bonizzoni *et al.*, 2013). However, resistance status of *A. albopictus* to temephos in the selected sentinel sites is still unknown, despite frequent application of temephos in the region over dengue outbreaks. Therefore, the present investigation was undertaken to assess the susceptibility/ resistance status of *Ae. albopictus* against organophosphorus temephos, in Chidambaram, Tamil Nadu, so as to provide a precise application rate of temephos against the targeted vector in sampled areas.

MATERIALS AND METHODS

The study was carried out in Chidambaram, Tamil Nadu, India (11° 23' 53.4984" N; 79°41'43.2888" E). Based on recent vicious dengue outbreaks in the area (Basker and Kolandaswasmy, 2015), *Ae. albopictus* larvae were collected from three different sentinel sites from Chidambaram-Town, Annamalai-Nagar, and Muthiah-Nagar. Sampling of the specimen was done two ways; a) Larvae were gently collected from their natural breeding habitats using a plastic dropper and dipper cup with a handy magnifying glass and transferred into a plastic cup as per the guidelines given in (WHO, 2016); and b) Ovitrap surveillance was conducted in the month of October 2021 as per (IAEA, 2017).

Specimen from each station was colonized until 1st generation (F1) and late 3rd instar larvae were used for the bioassay and susceptibility tests. The specimen was identified morphologically following the illustrated keys (Reuda, 2004) and then molecular identification was conducted at TRI-BIOTECH, Trichy Research Institute of Biotechnology Pvt. Ltd., Thillai-Nagar, Tamil Nadu, India (Soliang *et al.*, 2022). Samples of *Ae. albopictus* larvae and eggs (post-hatching) were maintained in allocated mosquito insectary at Department of Zoology, Faculty of Science, Annamalai University. Temperature and humidity of the colony were maintained following the methods (Govindarajan and Sivakumar, 2011) with temperature ranging between 27±3°C and relative humidity was kept at 70 - 80 per cent.

The larval specimens from each site were pooled and transferred into a larval tray of 40 x 30 x 8 cm in dimension. Larvae were fed on with larval diet, which consisted of pup-start (Puppy feed) and yeast in 60:40 ratios totalling 3g in 100 ml of water for a 500-1000 larvae population. Newly emerged adult was kept in a mosquito cage of 30 x 30 x 30 cm dimension and fed on sugar feed for 2-3 days post-emergence. Feeding was met with 10 per cent sucrose solution and overnight soaked raisins for better nourishment. Following sugar feed, before blood feed, one-day sugar feed abstinence was observed for a quality blood feed. The live mouse was exposed for a period of one hour per day for the next 2-3 days. Thereafter, whatman filter paper in a black cup with water occupying 1/2 of the cup was put in for oviposition. The eggs obtained are then hatched to produce F1 progeny. Third to fourth instar larvae were used for larval bioassay and susceptibility tests.

Temephos of organophosphate was selected for the present study due to its availability and as it is primary insecticide used for vector control. Technical grade temephos 50 per cent EC was sponsored by the Deputy Director of Health Service, Cuddalore, Tamil Nadu.

Baseline bioassay was conducted according to WHO standardized procedure (WHO, 2005; WHO, 2016) in the laboratory on late 3rd and early 4th instar stages. Technical grade temephos used had a 50 per cent efficacy concentration. Therefore, 2 ml of temephos was dissolved in one litre of double distilled water to yield a 1ppm stock solution. Following six discrete concentrations were chosen for the narrow range bioassay; 0.002 ppm, 0.003 ppm, 0.004 ppm, 0.005 ppm, 0.006 ppm and 0.007 ppm yielding between 30 to 100 per cent larval mortality in 24 h. Four replicates for each concentration were set up for treated and two replicates for control assays. Batches of 25 larvae were transferred with the help of a dropper into the disposable cups of 120 ml capacity. The test containers are held at 27±3°C and preferably a photoperiod of 12 h light followed by 12 h dark (12 L: 12 D). After 24 hours of exposure time, the larval mortality was recorded in standard test form made available by World Health Organization (WHO, 2005). Mortality of the larvae was detected by lightly stirring them with a clean plastic pipette. Moribund

larvae were counted as dead. The bioassay results were subjected to Probit Analysis (Finney, 1971), for lethal concentrations by using SPSS software V22 with significance value of 0.05. The resistance ratio (RR) was calculated based on the computed LC_{50}, LC_{90} and LC_{99} values, using the following formula:

$$\text{Resistance ratio (RR)} = \frac{LC_{50} / LC_{90} / LC_{99.9} \text{ of field strain}}{LC_{50} / LC_{90} / LC_{99.9} \text{ of laboratory strain}}$$

Guidelines of (Mazzarri and Georghiou, 1995) were used to classify the RRs as high (>10 fold), medium (between 5 and 10 fold) or low (<5 fold). Mortality correction through (Abbott, 1987) was not accounted as the pupated percentage and larval mortality in the test were negligible.

Susceptibility bioassay was conducted according to WHO (2005; 2016) to determine phenotypic resistance using discriminating or diagnostic concentrations drawn from the aforementioned baseline bioassay result. It is taken as double the concentration corresponding to 99.9% mortality (the $LC_{99.9}$ value), at which all the individuals in a susceptible population will be killed. This is conventionally known as the discriminating (or diagnostic) concentration (i.e., 1x). For each station, four replicates were taken for both treated and control samples with equal batches of larvae, i.e., 25 larvae of early 3rd and 4th instar stages. Unlike baseline bioassay, susceptibility assay is run for one hour. The discriminating concentrations used for Chidambaram-Town, Annamalai-Nagar and Muthiah-Nagar were estimated as 0.022 ppm, 0.024 ppm and 0.018ppm respectively. The data were interpreted following the guidelines of (WHO, 2016), which categorizes the result into three parts based on the susceptibility assay mortality percentage; i) Susceptible-larval mortality > 98 per cent; ii) Possible resistance- larval mortality 90-98 per cent; iii) Resistant-larval mortality < 90 per cent.

RESULTS AND DISCUSSION

Baseline bioassay: The larval bioassay result LC_{50} LC_{90} and $LC_{99.9}$ estimated for Chidambaram-Town were 0.003 ppm, 0.006 ppm and 0.011 ppm respectively. LC_{50} LC_{90} and $LC_{99.9}$ estimated for Annamalai-Nagar were 0.003 ppm, 0.007 ppm and 0.012 ppm respectively and LC_{50} LC_{90} and $LC_{99.9}$ estimated for Muthiah-Nagar were 0.002 ppm, 0.005 ppm and 0.009 ppm respectively. The resistance ratio (RR) in all the case was negligibly low with value much lower than 5 fold resistance ratio categorisation. Moreover outcome of the study was observed highly significant with statistical significant value of 0.000 ($P<0.05$ (Table 1).

Susceptibility bioassay: The susceptibility test serves as a tool to detect the existence of resistant vectors against any insecticide available in the public domain. Primary database required for the assay is discriminating concentration, which can be evaluated through a baseline bioassay. The result of susceptibility bioassay is illustrated in table 2, which indicates that vector population from the selected sites are still susceptible to on-going temephos, with mortality percentage of 98 for Chidambaram-Town and Annamalai-Nagar, and 100 for Muthiah-Nagar. According to insecticide resistance classification of WHO, *Aedes albopictus* larvae from Muthiah-Nagar were observed highly susceptible to temephos, while the specimen from Chidambaram-Town and Annamalai-Nagar are prompt to build resistance early.

Despite intense application of control measures, dengue vector population continued to dominate the public health (Mirresmailli and Isman, 2014). The main cause is interruption of vector control efficacy by insecticide resistance development (Meenambigai *et al.*, 2022) and lack of efficient drugs (Porretha *et al.*, 2022). Measures like application of insecticides in rotation manner and resistance management have been adopted to overcome incidence of resistance development (Araújo *et al.*, 2013; Morgan *et al.*, 2022). Moreover early detection of resistance ensures primary success of vector control measures. This is achieved by performing susceptibility bioassay (Reyes-Solis *et al.*, 2014) which can detect existence of resistant vector population and help in duly resistance management (Kraemer *et al.*, 2015).

Table 1. Bioassay of *Aedes albopictus* larvae against temephos from Chidambaram-Town; Annamalai Nagar and Muthiah Nagar, Tamil Nadu

SentinelSite	Conc. (ppm)	T	M%	`95% Confidential Interval (CI)			P Value
				LC_{50}(ppm) RR_{50}	LC_{90} (ppm) RR_{90}	$LC_{99.9}$(ppm) $RR_{99.9}$	
Chidambaram Town	0.002	100	40	0.003 [0.001-0.004]	0.006 [0.004-0.018]	0.0011 [0.007-0.118]	
	0.003	100	47				
	0.004	100	59				
	0.005	100	78	1.33	1.5	1.18	0.00
	0.006	100	96				
	0.007	100	100				
	Control	50	1				
Annamalai Nagar	0.002	100	30	0.003 [0.002-0.004]	0.007 [0.005-0.013]	0.012 [0.008-0.48]	
	0.003	100	42				
	0.004	100	59				
	0.005	100	70	1.67	1.14	1.17	0.00
	0.006	100	89				
	0.007	100	100				
	Control	50	2				
Muthiah Nagar	0.002	100	48	0.002 [0.001-0.003]	0.005 [0.004-0.008]	0.009 [0.006-0.028]	
	0.003	100	60				
	0.004	100	74				
	0.005	100	89	1.5	1.2	1.11	0.00
	0.006	100	99				
	0.007	100	100				
	Control	50	2				

Conc. (Concentration); T (Total number of exposed larvae to temephos for 24 hours); M% (Mortality percentage: ratio of total death divided to total number of larvae exposed multiplied by 100); RR(Resistance ratio: ratio of lethal concentration of field population to lab population); LC_{50} (Lethal concentration that kills 50% of the exposed larvae); LC_{90} (Concentration that kills 90% of the exposed larvae); $LC_{99.9}$ (Concentration that kills 99.9% of the exposed larvae); P (Statistical significance, which was found to be highly significant with $p<0.05$)

Diagnostic concentration or discriminating concentration is prerequisite data required for resistance surveillance and it differs widely from one station to another. In the present study also, though the sentinel sites are under same taluk, their discriminating concentration varied widely (Table 2), where the discriminating concentration for Chidambaram-Town, Annamalai-Nagar and Muthiah-Nagar were 0.022 ppm, 0.024 ppm, and 0.018 ppm respectively. Diagnostic concentrations

are formulated from lethal concentration, which are obtained through baseline bioassay (WHO, 2016; 2005). It is estimated as double of $LC_{99.9}$ (WHO, 2016). The $LC_{99.9}$ obtained in the current study for Chidambaram-Town, Annamalai-Nagar and Muthiah-Nagar was 0.011 ppm, 0.012 ppm and 0.009 ppm respectively. Findings of the study revealed that *Ae. albopictus* larvae are still susceptible to temephos in Chidambaram-Town, Annamalai-Nagar and Muthiath-Nagar with 98 mortality percentage.

Ae. albopictus is cosmopolitan (Romiti *et al., 2022;* Sivasankaran *et al., 2022)* and most invasive vector (Vanlandingham *et al.*, 2016), alarming public health concern with its ability to cause 32 proven pathogen diseases, subsuming dengue, chikungunya, and zika (Goubert *et al.,* 2016; Liu *et al.,* 2022; Morgan *et al.,* 2022a,b). Incidence of *Ae. albopictus* was observed at Arupathi (Mayiladuthurai district, Tamil Nadu, India) and Sityan-Gam (Lohit district, Arunachal Pradesh, India) in addition to the selected sentinel sites for the study. Like overseas countries (Bharati and Saha, 2021), in India also, temephos is specifically subjected to control of dengue vector larvae (Ocampo *et al*, 2011; Romiti *et al.*, 2022) and it has led to development of resistance (Singh *et al.*, 2014; Yadav *et al.*, 2015; Wu *et al.*, 2022). Tamil Nadu state lies in tropical climate zone and is endemic to DENV and to other vector borne disease as well (Shimono *et al.*, 2021; Lesmana *et al.*, 2022). Resistant dengue vector population to temephos are reported from the state (Fatima and Syed, 2018). The vaccines for dengue are made available but failed to gain public attention due to their low efficacy and hope for a reliable vaccine is still a long wait (Rai *et al.*, 2020; Hassan *et al.*, 2021). Vector control with chemical measures continues with timely resistance surveillance. Thus the present study provides effective vector control in the present scenario with precise kill using new formulated lethal concentrations (Table 1) and it sets primary database for monitoring *Ae. albopictus* resistance status in Chidambaram-town, Annamalai-Nagar and Muthiah-Nagar. With $LC_{99.9}$ value of 0.012 ppm, Annamalai-Nagar showed to have highest lethal concentration amongst the three selected stations and has potential to develop resistance early. LC_{50} and mortality percentage value of Annamalai-Nagar and Chidambaram-Town were found to be same, this could be due to close proximity of the stations sharing similar environment. Station Muthiah-Nagar showed to have the least lethal concentrations and cent per cent mortality indicating highly susceptible. Similar studies are conducted in different parts of the country where temephos is used as primary chemical control measure and the results are reported resistant (Sivan *et al.*, 2015). The current study yielded LC_{50} and RR_{50} of all the selected stations much lesser than that of (Sivan *et al.*, 2015) findings with RR_{50} of 15.3 and LC_{50} of 1.177ppm. In addition to source reduction vector control measures, susceptibility and resistance status surveillances have become the key point in today's vector control planning. Present investigation on insecticide resistance proved, *A. albopictus* larvae from the selected sentinel sites are susceptible to temephos. However, it is important to limelight, the specimen from Chidambaram-Town and

Table 2. Analysis of phenotypic resistant via susceptibility bioassay with the application of discriminating concentration (1x) against *Aedes albopictus* larvae (n=100)

Population Strain	1x (ppm)	Mortality%	Status
Chidambaram-Town	0.022	98	Susceptible
Annamalai-Nagar	0.024	98	Susceptible
Muthiah-Nagar	0.018	100	Susceptible

Mortality percentage with exposure period of one hour; Discriminating concentration (1x) is double the concentration of $LC_{99.9.}$

Annamalai-Nagar are likely to build resistance speedily. This study is the first insecticide resistance case study on *Ae. albopictus* resistance status against temephos in the selected sentinel sites. Findings of present investigation revealed that the vector species is still susceptible to on-going application of temephos. However, due and periodic resistance surveillance in the future is highly advised with the present results as baseline database.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Zoology, Annamalai University, Annamalai Nagar, Tamil Nadu, India for providing a suitable laboratory to carry out this studious study and to the Deputy Director of Health Service (DDHS), Cuddalore District, Tamil Nadu, and India for sponsoring the temephos.

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(Received September 01, 2022; revised ms accepted November 11, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.792
ENTOMON 47(4): 391-396 (2022)
Article No. ent. 47405

Relative efficacy of selected insecticides to check rice yellow stem borer *Scirpophaga incertulas* (Walker) (Lepidoptera, Crambidae) at Hooghly, West Bengal, India

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ABSTRACT: Rice yellow stem borer (YSB), *Scirpophaga incertulas* Walker is one of the major destructive insect pests rendering huge crop damage. Nine insecticide formulations, either solely or in combinations were applied in the rice (*var. Lalat*) field for two consecutive seasons during 2019-2021 to assess their efficacy to suppress YSB population and to stabilize yield. The combination of flubendiamide (480 SC) @80 g a.s.ha^{-1} on 45 DAT and deltamethrin (1%) + triazophos (35%) @300 g a.i.ha^{-1} on 80 DAT, treated the rice crop, recorded minimum YSB incidence (4.14 egg clutches, 4.78 larvae and adults 3.17/5 hills) and damage (2.12% dead hearts (DH) and 1.47 white ear (WE). This treatment gave significantly higher grain yield (3.63 t ha^{-1}), an increase of 45.78 per cent over control. The incidence (12.21 egg clutches, 14.12 larvae and adults 11.76/5 hills) and crop damage (14.83 DH and 11.10% WE) was maximum in the treatment, neem seed kernel extract (5%) @50 ml L^{-1} at 15- day intervals after transplanting and neem leaf extract (5%) @7 ml a.s. L^{-1} on 35, 50, 65 and 80 DAT, resulting in minimum yield (2.88 t ha^{-1}). Other combinations of insecticide application gave variable results.

KEY WORDS: Grain, yield, damage, population, flubendiamide, deltamethrin, triazophos

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal crop and primary energy source for two third of the world's population (Khan *et al.*, 2015). India ranks first in area of cultivation and second in rice production in the world (DES, Govt. of India, 2016). Annually, about 30 per cent pre-harvest crop loss was noted in India (FAO, 2018). Out of that, insect pests cause, in average, 25-41 per cent rice crop damage, globally (Savary *et al.,* 2019). Rice yellow stem borer (YSB), *Scirpophaga incertulas* Walker is the most dominating and destructive insect pest that ravages the rice field globally. To check insect pest induced crop damage, Indian farmers apply insecticides of different newer brands in high quantum without any concern to the environment and also to the farmer's health (Horrigan *et al.*, 2002). Under modern IPM practice, the best way to reduce pesticide 'tread-mill' is to rely on phyto-formulation based pest control methods (Watts, 2010). Relative efficacy of nine selected insecticide formulations was evaluated against YSB.

MATERIALS AND METHODS

The experiment was carried out at paddy field area of Tarakeswar, Hooghly (22.8958° N; 88.0159°E) in two consecutive *kharif* seasons during 2019-2021. The rice cultivar *Lalat* (IET-9947), a most widely grown popular rice variety was used for the experiment. Parentage of this cultivar were

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Obs.677×IR-207×Vikram rice varieties.

The seedbeds measuring 20 m×1 m were prepared at about 26 standard meteorological weeks (SMW) in each year. Pre-germinated rice seeds of the cultivar *Lalat* (IET-9947) were sown at 28 SMW in the main land that was prepared following conventional management practice. Nine plots each measuring about 25 m×20 m were prepared. All of the plots were separated by a clear space of 5m from the nearby plot. Triple super phosphate, muriate of potash, gypsum and zinc sulphate fertilizers were applied at the rate of 220 kg ha^{-1} in each plot in three equal splits at 15, 30 and 50 days after seedling transplantation (DAT) respectively. Forty day-old seedlings of *Lalat* rice were transplanted at 20 cm × 20 cm spacing in all of the nine plots at about 28 SMW. Conventional cultural practices were accomplished for all of the plots. Seedling was transplanted equidistantly with fixed row-row and hill-hill spacing. Both organic (6.5 t decomposed cow dung ha^{-1}) and inorganic N (120 urea kg ha^{-1}) fertilizer were applied; inorganic fertilizer to rice field was given in two equal splits *i,e.* during vegetative and early reproductive growth stage, fortnightly light trapping of adult YSB population, alternation wetting and drying at 7-day interval from 60 days after seedling transplantation (DAT) was adopted. Periodic field scouting for the dead and old leaves for all treatments including the check (control) was followed.

Preparation of bio-formulations:

i. 150 g of 3 months old neem kernel is finely smashed and subsequently pounded in 1 litre of hot water (1:1 w/v) to prepare neem seed kernel extract (NSKE) formulation.

ii. Neem oil obtained through pressing or crushing of the dry seed kernel. Neem oil 15-30 ml is added to 1 litre of water and stirred well. To this emulsifier stearyl amine ethoxylates is added (1ml L^{-1}).

iii. Similarly, 1 kg green neem leaves were soaked overnight in 5 litre of water, then grinded and the leaf extract was filtered to prepare neem leaf extract (NLE) formulation. Extract solution was kept in the shade for a day and subsequently sieved to get a clear extract of stock solution. From the stock solution workable solution grade was prepared.

Preparation of synthetic insecticide-formulations: During the selection of synthetic insecticides, broad-spectrum hazardous insecticides were generally avoided. But selection was done aiming to replace the conventionally applied highly toxic insecticide by relatively less toxic and eco-friendly formulation. There were seven synthetic insecticides and three neem formulations in the experimental evaluation. Nine treatments were formulated with synthetic insecticides and neem formulations along with an untreated check (Table1). Four replications for each treatment were done. The treatments were applied as in the Table 1. YSB damage was recorded in terms of per cent of dead hearts (DH) and per cent white ears (WE) produced during vegetative and reproductive growth stages of rice plant respectively in each plot. The percentage of DH and WE of individual plot was calculated by using the following formula -

$$\text{DH / WE \%} = \frac{\text{Number of DH or WE/hill}}{\text{Total number of tillers/hill}} \times 100$$

The population of egg clutches, larvae and adults of YSB was recorded on 20 randomly selected hills from each plot were at seven day intervals after seedling transplantation. Grains from each plot were dried and weighed. Collected data were subjected to pooled analysis of variance, with square root transformed and compared on the basis of Duncan's Multiple Range Test (DMRT) using SPSS-ANOVA software.

RESULTS AND DISCUSSION

All the insecticide formulations were effective in suppressing the YSB infestation significantly compared to untreated control. But considerable variation in the relative efficacy among the insecticidal treatments was noted.

Assessment based on YSB egg clutches: YSB eggs are laid in groups and each group is called

Table 1. Dose of insecticide and time of application under different treatments

No.	Treatments with dose and time of application
T1	Flubendiamide (480 SC) @80 g a.s./ha on 45 DAT and deltamethrin (1%) + triazophos (35%) @300 g a.i. ha L^{-1} on 80 DAT
T2	Rynaxypyr (0.4% G) @ 50g a.s. ha L^{-1} on 45 DAT and chlorpyriphos (50%) @0.5 kg a.i ha L^{-1} on 75 DAT
T3	Chlorpyriphos (50%)+ organophosphate+ cypermethrin (5%) @ 2 ml a.s. L^{-1} on 35 DAT and carbofuran (35 G) @ 5 g a.s./plant on 50 DAT
T4	Carbofuran (35G) @ 12 g a.s. ha L^{-1} on 35 DAT and NLE (5%) @7 ml a.s. L^{-1} on 45 and 65 DAT
T5	Neem oil @50 ml L^{-1} on 20 DAT and chlorpyriphos (50%)+ organophosphate + cypermethrin (5%) @2 ml a.s. L^{-1} on 35 DAT
T6	NSKE (5%) @7 ml a.s. L^{-1} on 20 DAT and flubendiamide (480 SC)@ 80 g a.s. ha^{-1} on 45 DAT
T7	NSKE (5%) @7 ml a.s. L^{-1} on 20 DAT and neem oil @50 ml L^{-1} on 30, 45, 65 and 75 DAT
T8	Neem oil @50 ml L^{-1}on 20 DAT and NLE (5%) @7 ml a.s. L^{-1} on 35, 50, 65 and 80 DAT
T9	NSKE (5%) @50 ml L^{-1} at 15 day intervals after transplanting and NLE (5%) @7 ml a.s. L^{-1} on 35, 50, 65 and 80 DAT
T10	Untreated (Control)

DAT- Days after seedling transplantation; a.s.- active substance; a.i.- active ingredient

egg clutch. A mixture of flubendiamide (480 SC), deltamethrin (1%) and triazophos (35%) (T1) treatment showed 4.14 YSB egg clutches/5 hills, whereas in the rynaxypyr (0.4% G) and chlorpyriphos (50%) (T2) application there were 5.20 egg clutches. In the neem oil, chlorpyriphos (50%), organophosphate and cypermethrin (5%) (T5) treated plots, 5.93 egg clutches were noted. The treatment of a mixture of carbofuran (35 G), chlorpyriphos (50%), organophosphate and cypermethrin (5%) (T3) recorded 6.12 egg clutches. Carbofuran (35G) and NLE (5%) (T4) when applied jointly, 6.92 egg clutches were noted. Flubendiamide (480 SC) and NSKE (5%) (T6) resulted in 7.10 egg clutches. Combination of NSKE (5%) and neem oil (T7) had 10.12 egg clutches. In neem oil and NLE (5%) (T8) applied plots 11.29 egg clutches were noted. There were 12.21 egg clutches in the NSKE (5%), NLE (5%) (T9) treated plots. Whereas, untreated control field (T10) has registered highest 16.31 egg clutches (Table2).

Assessment based on YSB incidence (individuals/5 hills): A mixture application of flubendiamide (480 SC), deltamethrin (1%) and triazophos (35%) (T1) recorded 4.78 larvae and 3.17 adult YSB/5 hills. This was followed by (T2) rynaxypyr (0.4% G) and chlorpyriphos (50%) application with 5.62 larvae and 3.67 adults. In the treatment T5 (neem oil, chlorpyriphos (50%), organophosphate and cypermethrin (5%) combination) the incidence was 5.93 larvae and 4.06 adults. This was followed by T3 (6.74 and 4.55), T4 (7.09 and 4.95), T6 (7.89 and 5.10), T7 (12.29 and 9.29), T8 (13.34 and 10.38) and T9 (14.12 and 11.76) in ascending order. Whereas, untreated control field has registered 18.42 larvae and 15.89 adults (Fig. 1, Table 2).

DH and WE (%): There were significant variations in the DH and WE among the treatments. The treatment T1 showed a minimum damage of 2.12 per cent DH and 1.47 per cent WE and the

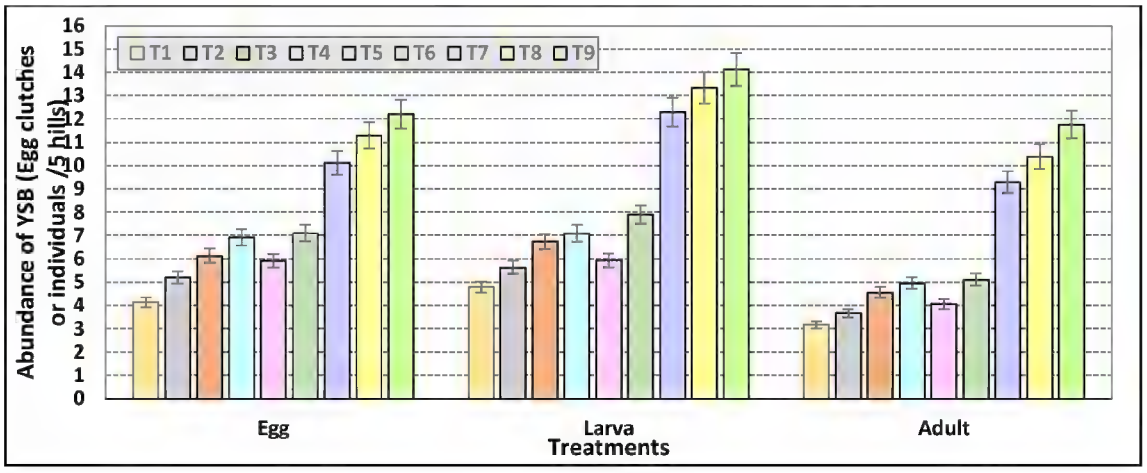

Fig. 1 Effect of different treatments on the incidence of *S.incertulas*

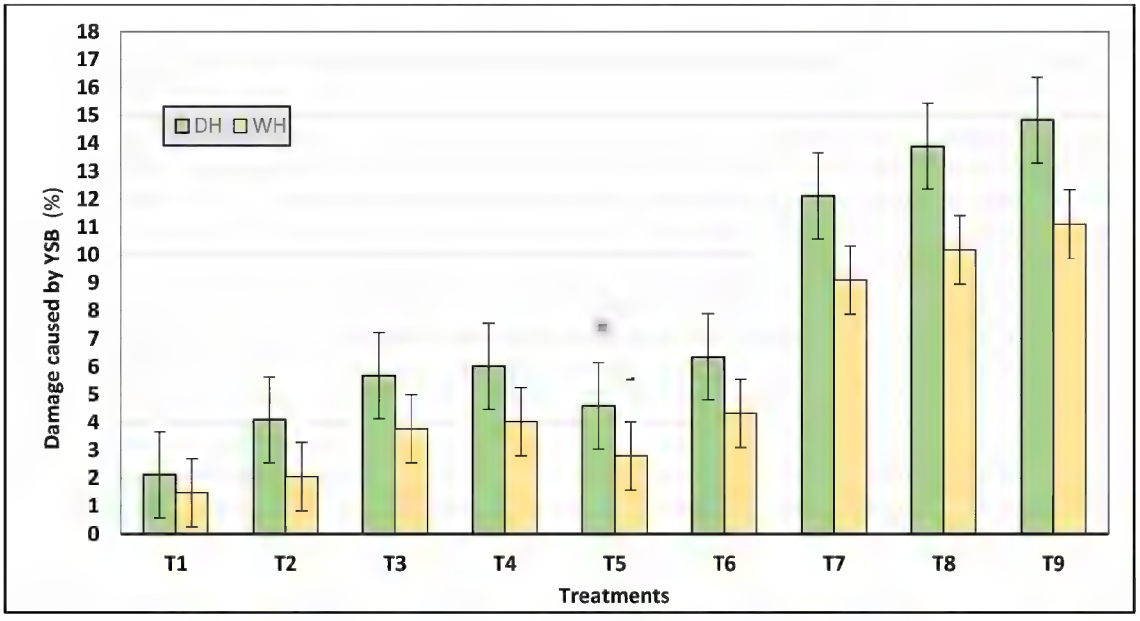

Fig. 2 Effect of different treatments on the extent of damage by *S.incertulas*

maximum was noted in T9 (14.83 DH; 11.10 WE). DH and WE in rest of the treatments were intermediate in nature and the values are T2 (4.09 DH; 2.05 WE), T3 (5.67 DH; 3.77 WE), T4 (6.01 DH; 4.02 WE), T5 (4.59 DH; 2.79 WE), T6 (6.34 DH; 4.33 WE), T7 (12.12 DH; 9.09 WE) and T8 (13.89 DH; 10.18 WE); whereas the control field showed highest damage with 18.74 per cent DH and16.79 per cent WE (Fig. 2, Table 2).

Effect of treatments on yield: Maximum yield benefit with least YSB damage was noted in T1 and was significantly higher than other treatments. Application of flubendiamide (480 SC), deltamethrin (1%) and triazophos (35%) (T1) recorded significantly higher yield (3.63 t ha^{-1}). This was followed by the treatments - T2 (3.55 t ha^{-1}), T5 (3.49 t ha^{-1}), T3 (3.37 t ha^{-1}), T4 (3.32 t ha^{-1}), T6 (3.20 t ha^{-1}), T7 (3.08 t ha^{-1}), T8 (2.99 t ha^{-1}) and T9 (2.88 t ha^{-1}). In untreated control (T10) the grain yield was 2.49 t ha^{-1}. Extent of yield generation over the check was highest in T1 (45.78%) that was followed by T2 (42.25%), T5 (40.04%), T3 (35.27%), T4 (33.11%), T6 (28.56%), T7 (23.44%),

T8 (19.86%) and T9 (15.62%) respectively in descending order (Table 2).

In the present study all the insecticide formulations were found effective to suppress YSB population in consideration of untreated control. But considering all aspects of the treatment, a mixture application of flubendiamide (480 SC), deltamethrin (1%) and triazophos (35%) (T1) showed minimum YSB population and damage with maximum yield in comparison to the other treatments. This was followed by the mixture of rynaxypyr (0.4% G) and chlorpyriphos (50%) (T2), neem oil, chlorpyriphos (50%), organophosphate and cypermethrin (5%) (T5), a mixture of carbofuran (35 G), chlorpyriphos (50%), a mixture of organophosphate and cypermethrin (5%) (T3), combination of carbofuran (35G) and NLE (5%) (T4), a mixture of flubendiamide (480 SC) and NSKE (5%) (T6), combination of NSKE (5%) and neem oil (T7), neem oil and NLE (5%) (T8) and NSKE (5%), NLE (5%) (T9) respectively. There was no significant difference between the efficacy of a mixture of organophosphate and cypermethrin (5%) (T5) with a combined application of flubendiamide (480 SC), deltamethrin (1%) and triazophos (35%) (T1). Application of rynaxypyr (0.4% G) and chlorpyriphos (50%) (T2) had somewhat similar result. Combination of organophosphate and cypermethrin (5%) (T5) has less effect. Whereas combination of NSKE (5%) and neem oil (T7) , neem oil and NLE (5%) (T8) and NSKE (5%), NLE (5%) (T9) were purely botanical in nature, but their effectiveness against YSB and in crop

Table 2. Effect of the treatments on the incidence, infestation and damage of *Scirpophaga incertulas*

Treatment	*S. incertulas* population/5 hills			Extent of damage (%)		Yield (t ha^{-1})	Increase (%)
	Egg clutches	Larvae	Adult	DH	WE		
T1	(2.03) 4.14^{b}	(2.18) 4.78^{b}	(1.78) 3.17^{ab}	(1.62 2.12^{a}	(1.40) 1.47^{a}	3.63^{e}	45.78
T2	5.20^{b} (2.28)	5.62^{b} (2.37)	3.67^{ab} (1.91)	4.09^{b} (2.14)	2.05^{a} (1.60)	3.55^{d}	42.25
T3	6.12^{c} (2.47)	6.74^{c} (2.59)	4.55^{b} (2.13)	5.67^{b} (2.48)	3.77^{ab} (2.07)	3.37^{c}	35.27
T4	6.92^{c} (2.63)	7.09^{c} (2.66)	4.95^{bc} (2.22)	6.01^{c} (2.55)	4.02^{b} (2.13)	3.32^{c}	33.11
T5	5.91^{b} (2.43)	5.93^{b} (2.43)	4.06^{b} (2.01)	4.59^{b} (2.26)	2.79^{a} (1.81)	3.49^{a}	40.04
T6	7.10^{d} (2.66)	7.89^{d} (2.80)	5.10^{c} (2.25)	6.34^{c} (2.62)	4.33^{b} (2.20)	3.20^{c}	28.56
T7	10.12^{de} (3.18)	12.29^{ef} (3.50)	9.29^{de} (3.04)	12.12^{ef} (3.48)	9.09^{de} (3.01)	3.08^{b}	23.44
T8	11.29^{de} (3.36)	13.34^{f} (3.65)	10.38^{de} (3.22)	13.89^{f} (3.72)	10.18^{de} (3.19)	2.99^{ab}	19.86
T9	12.21^{ef} (3.49)	14.12^{f} (3.75)	11.76^{de} (3.42)	14.83^{f} (3.85)	11.10^{de} (3.33)	2.88^{ab}	15.62
T10 (control)	16.31^{h} (4.03)	18.42^{i} (4.29)	15.89^{g} (3.98)	18.74^{i} (4.32)	16.79^{h} (4.09)	2.49^{a}	------

Figures in parentheses are the square root transformed values; Means followed by same letters in the column do not differ significantly by DMRT (p=0.05)

yielding was not significant in comparison to the other treatments. While a mixture of carbofuran (35 G), chlorpyriphos (50%), organophosphate and cypermethrin (5%) (T3), carbofuran (35G) and NLE (5%) (T4) and flubendiamide (480 SC) and NSKE (5%) (T6) showed average effect in comparison to the others.

In consonance to the present observation Jagginavar *et al.* (2009) have reported that fluben diamide was highly effective against lepidopteran insect pests of rice. It has also been documented that flubendiamide was comparatively safe to natural enemies but suppressed lepidopteran pests population effectively (Hall *et al.,* 2007). Rynaxypyr 0.4G @ 40 and 50 g a.i. ha^{-1} could effectively control stem borer complex and increasing rice grain yield (Kandasamy *et al.,* 1986). In parity to the present observation Ho and Kibuka (1983) reported that neem oil can control borer menace at vegetative stage. Application of 3 per cent neem oil could effectively suppress YSB as suggested by Nanda *et al.* (1996) and Murugabharathi *et al.* (1999). In parity to the earlier observation by Nanda *et al.* (1996), in the present experiment it was found that NSKE moderately effectively suppressed rice borer. Ahmed *et al.* (2002) has stated that neem formulations were economically prudent to suppress stem borer menace like the present experiment. Sasmal *et al.* (2010) reported that neem formulation moderately suppressed white head in the rice cultivar Jaya in Orissa.

ACKNOWLEDGEMENT

The authors full heartedly acknowledge the support of the faculty members of the concerned departments from whom guidance and suggestions were taken as and when needed to carry out the experiment.

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(Received June 07, 2022; revised ms accepted October 01, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.793
ENTOMON 47(4): 397-414 (2022)
Article No. ent. 47406

New records of Halictini (Hymenoptera, Halictidae, Halictinae) from Manipur, India

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ABSTRACT: Distributional records of the Halictinae bees of the genus *Halictus* (the subgenus *Seladonia), viz., H. lucidipennis* Smith, *H. propinquus* Vachal, genus *Lasioglossum* (the subgenus *Ctenomia*) *albescens* (Smith, 1853), *L. cavernifrons* Bluthgen, 1926, *L. sikkimense* (Blüthgen, 1926), *L. splendidulum* (Vachal, 1895), *L. vagans* (Smith, 1857) and genus *Patellapis* (*Pachyhalictus*) *liodoma* (Vachal, 1895), *P. reticulosa* (Dalla Torre, 1896) from North-East India, Manipur are listed. Re-described the female specimen, along with the collection site. © 2022 Association for Advancement of Entomology

KEY WORDS: Taxonomy, redescription, female specimen, distributional records

INTRODUCTION

As it is known that bees play an important role in the pollination of angiosperms, and the members of Halictidae also have great influence in this service. Halictidae is the second largest group of bees, with approximately 4,510 recognized species worldwide (Ascher and Pickering, 2022). Four subfamilies are recognized under Halictidae (Michener, 2007); Rophitinae Schenck, 1866; Nomiinae Robertson, 1904; Nomioidinae Börner, 1919; and Halictinae Thomson, 1869. Halictid bees make their nest in the soil or rarely in rotting wood; and have a very diverse social structure like eusocial, semi social, solitary and communal (Michener, 1978; Schwarz *et al.,* 2007). Some of genera and species in Halictidae are kleptoparasites. In the Asia region, Halictidae family is common, often dominating other bee families in number of species and individuals. The Halictini is the largest tribe of Halictidae having more than 1600 species, within the subfamily of sweat bees (Halictinae), under 23 genera *sensu* Michener (2007).

The bee Subgenus *Seladonia* Robertson of subfamily Halictinae has 75 recognized species (Ascher and Pickering, 2022). According to both molecular and morphological phylogenetic analyses (Pesenko and Davydova, 2004; Danforth *et al.,* 1999; Gibbs *et al.,* 2012), this genus is the sister group to the genus *Halictus* Latreille. Subgenus *Seladonia* differs from *Halictus* by the body having a metallic green or blue-green luster, posterior margin of fourth metasomal sternum straight and male genitalia with medial lobe on upper gonostylus. We treat *Seladonia* at the Sub generic level in this study, in accordance with (Michener, 2007). The Genus *Lasioglossum*

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Curtis is highly diverse group of bees with approximately 1881 species worldwide (Ascher and Pickering, 2022). The main character of this genus is the fore wing with weakened 2r-m and 2m-cu veins in female. *Lasioglossum* is classified into two groups (Michener, 2007): (1) the *Hemihalictus* Cockerell series (weak-veined *Lasioglossum*), which includes all subgenera with weak second sub marginal vein (1rs-m) of the female fore wing; and (2) the *Lasioglossum* series (strong-veined *Lasioglossum*) which includes all subgenera with strong second sub marginal vein (1rs-m) of the female fore wing. The subgenus *Patellapis* (*Chaetalictus*) comprises 46 species and has recently been revised (Timmermann and Kuhlmann, 2008 a, b). The name *Patellapis* was first used by Friese (1909) proposing a subgenus *Patellapis* for a group of black *Halictus* found in South Africa, characteristic for having a large rounded apical plate on the abdomen of the male.

Unfortunately, North East India has little available data of Halictid bees. Only a few common species have been documented by Smith (1853), Vachal (1895), Bingham (1897) and Bluthgen (1925). A few new species from other North East Indian states have been published like *Halictus lucidipennis* Smith, 1853 and *Halictus propinquus* Smith, 1853 from Assam, *Halictus subauratoides* Blüthgen, 1925 from Meghalaya and some species of genus *Lasioglossum* by other international authors. However, till date no reports on halictid bees from Manipur exist. Therefore, it was crucial to investigate the Halictid bee fauna of Manipur. The present study aims to revise the Halictidae species of North East India.

MATERIALS AND METHODS

The specimens studied here belong to the Tribe Halictini of subfamily Halictinae which were deposited in the National Pusa Collection (NPC), ICAR – IARI, New Delhi, India. The specimens were brought to the laboratory, suitably processed according to established procedures for further studies. Identification was done by the literature (Sakagami, 1989; Bluthgen, 1925; Sakagami *et al.*, 1996; Michener, 2007). Photography was with Leica Stereo Zoom Microscope M205 FA fitted with digital camera Leica DFC425 C. Terminology mainly follows Michener (1978, 2007), Bluthgen (1925) and Sakagami *et al.* (1989).

Description of the collection site**:** Manipur is a state in northeastern India and bounded by the Indian states of Nagaland to the North, Mizoram to the south and Assam to the west. The state lies at a latitude of 23°83'N – 25°68'N and a longitude of 93°03'E – 94°78'E. The state covers an area of 22,327 square kilometers (8,621 sq miles). Collection sites are Krishi Vigyan Kendra (KVK) farm Ukhrul, ICAR Research Complex for NEH Region, Kamong, Sangaithel area, and Langol ICAR farm Manipur, India.

Abbreviation used: Body Length (BL) (from Clypeus margin to metasomal tip), Head length (HL), Head Width (HW), Eye Length (EL), Wing Length (WL), Inter Ocellar Distance (IOD),

Gena Length (GL), Gena Width (GW), Clypeus Length (CL), Clypeus Width (CW), Abdomen Length (AL), Abdomen Width (AW).

RESULTS AND DISCUSSION

Subfamily Halictinae

Genus I- *Halictus*

***Halictus* (*Seladonia*) *lucidipennis* Smith, 1853 (Figs. 1-6)**

Halictus (*Seladonia*) *lucidipennis* Smith, 1853: 362; Ember, 1980: 483.

Halictus varipes Morawitz, 1876: 223-224; Sakagami and Ember, 1987: 326, pauly 1999: 146

Halictus vernalis Smith, 1879: 30

Halictus niloticus Smith, 1879: 32

Halictus magrettii Vachal, 1892: 137.

Halictus dives Perez, 1895: 52.

Halictus omanicus Perez, 1907: 489.

*Halictus variipes*var.*koptica* Blüthgen, 1933: 16.

Halictus (*Seladonia*) *sudanicus* Cockerell, 1945: 352.

Halictus (*Seladonia*) *tokarensis* Cockerell, 1945: 352.

Figs. 1-6 Female. *Halictus* (*Seladonia*) *lucidipennis*, 1-Dorsal habitus; 2- Lateral habitus; 3- Head; 4- Thorax; 5- Propodeum; 6-Hind tibial spur teeth

Figs. 7-10 Male. *Halictus* (*Seladonia*) *lucidipennis*, 7 –Dorsal habitus; 8- lateral habitus; 9- Sternum; 10-Head

Halictus (*Seladonia*) *dissensis* Cockerell, 1945: 353

Halictus (*Seladonia*) *medanicus* Cockerell, 1945: 354.

Halictus (*Seladonia*) *mogrensis* Cockerell, 1945: 355.

Halictus (*Seladonia*) *tokariellus* Cockerell, 1945: 355.

Halictus (*Seladonia*) *medaniellus* Cockerell, 1945: 356.

Halictus (*Seladonia*) *morinellushyemalus* Warncke, 1982: 134.

Halictus (*Seladonia*) *lucidipennis* (Smith, 1853: 362); Sakagami and Ember, 1987: 321; Pesenko, 2004: 101.

Diagnosis: *Halictus* (*Seladonia*) *lucidipennis* can be distinguished from other *Seladonia* species by the following: small size, fine punctures dorsally; tegula sparsely punctured anteriorly, basal propodeal with longitudinal ridges reaching up to mid-length only.

Coloration: Generally pale, non-metallic parts rather brownish; flagella ventrally dark brown; tegula semi-transparent, pale brown; legs chestnut brown. Base of fore and mid tibiae yellow or sometimes pale brown; fore tibia and tarsi, apices of mid tibia and hind femur, and base and apex of hind tibia pale brown; mid and hind tibiae pale chestnut brown.

Structure: BL- 6.19mm

Head (Fig. 3): distinctly wider than mesosoma and metasoma; HW- 1.72mm HL- 1.43mm;CL- 0.31mm, CW- 0.56mm, IOD- 0.58mm; vertex flat and sometimes faintly concave medially; frons mildly but distinctly convex, frontal carina relatively long;clypeus sub apically slightly depressed gently rosebelow; marginal area strongly depressed; hypostoma very sparsely and finely punctured.

Mesosoma (Fig. 4): pronotum with lateral ridge acute but not extending below; lateral surface coriaceous and shagreen, below striated with dull vertically or obliquely paralleled ridges, much weaker than in *H. propinquus;* puncture on mesoscutum and scutellum homogeneous; propodeal dorsum with enclosure mildly depressed (Fig. 5); ridges occupying only anterior 1/2 to 2/3; medially ridges parallel but often slightly irregular; lateral field rather broadly impunctate and finely coriaceous and shining; tegula with anterior hairs short, punctures fine and sparse; post outer area broadly smooth.

Metasoma (Fig. 1): shiny; elongate and oval; apical hair band present T1- T5; T1 smooth with very fine and sparse punctures, T2 sparsely punctuate, T3 and T4 moderately punctuate; pygidial plate U-shaped; T2 to T5rough compared to T1;well developed scopa; basitibial plate oval, pointed apically; Inner hind tibial spur with3-4 relatively long and round-tipped teeth.

Male – BL- 8.19 mm (Figs. 7- 10)

More slender than female, same coloration and punctuation; head is longer than female; flagella ombre yellow, pale.

Flower record: Marigold

***Halictus* (*Seladonia*) *propinquus* Smith (Figs. 11-16)**

Halictus propinquus Smith, 1853, 1: 60-61.

Halictus grandiceps Cameron, 1896, 41(4): 98-99.

Halictus alexis Cameron, 1896, 41(4): 99-100.

Halictus pinguis Vachal, 1902, 2: 230.

Halictus propinquss Smith: Michener, 1978, 51(16): 528.

Halictus propinquus Smith: Ebmer, 1980, 12(2): 481.

Halictus (*Seladonia*) *propinquus* Smith: Sakagami and Ebmer, 1987, 19(2): 321.

Halictus (*Seladonia*) *propinquus* Smith: Ebmer, 1988, 68(4/6): 345.

Halictus (*Seladonia*) *propinquus* Smith: Fan, 1991, 34(4): 479- 480.

Halictus (*Seladonia*) *propinquus* Smith: Dawut and Tadauchi 2001, 41: 167-169.

Diagnosis: *Halictus* (*Seladonia*) *propinquus* can be distinguished from other *Seladonia* species by the following: sizelarger than *H. lucidipennis*, moderate punctures dorsally; tegula punctured anterior to mid-length, basal propodeal with reticulation reaching up to mid-length or up to Propodeal ridge.

Coloration: Generally darker, non-metallic parts are dark brown; flagella ventrally dark brown; pronotum lobe apically dark brown to blackish; tegula black brown anteriorly; legs dark brown to blackish. Base of fore and mid tibiae are brown, rarely yellowish.

Structure: BL- 7.55mm

Head (Figs. 13): as wide as mesosoma and metasoma. HW- 1.71mm HL- 1.51mm;CL- 0.42mm, CW- 0.68mm, IOD- 0.38mm vertex flatter not concave medially; frons mildly convex only; frontal carina variable long but shorter than in *H. lucidipennis.* paraocular area with dull epistomal angle; supraclypeus same as in *H. lucidipennis* but sparsely punctured; hypostoma finely punctured.

Mesosoma (Fig. 14): pronotum with dull lateral ridge; lateral surface coriaceous and shagreen,

below striated with strong vertically or obliquely paralleled ridges; irregular puncture on mesoscutum and scutellum; mesoscutellum medially not depressed longitudinally; propodeal dorsum ridges reaching up to edge (Fig. 15); lateral propodeal field smooth and shining with rather sparse punctures; tegula with long anterior hairs, punctuation denser than *H. lucidipennis.*

Metasoma (Fig. 11): less shiny; elongate and oval; denser punctuation; apical hair bands present T1-T5; T1 dull with sparse punctures, T2 sparsely punctuate, T3 & T4 moderately punctuate; pygidial plate U-shaped; well developed scopa; basitibial plate oval, pointed apically; Inner hind tibial spur with 4 – 6 small teeth. (Fig. 16).

Male – Unknown

Flower record: Rose, dahlia, cauliflower

Genus II – *Lasioglossum*

***Lasioglossum* (*Ctenomia*) *albescens* (Smith, 1853) (Figs. 17-22)**

Halictus albescens Smith, 1853:61.

Halictus albozonatus homonym Smith, 1879:32.

Halictus senescens (Smith, 1879:30); Vachal, 1895: 430.

Halictus albicinctus Dalla Torre, 1896:52.

Halictus picipes homonym Cameron, 1897:102.

Halictus minikoiensis Cameron, 1902a:58.

Halictus bengalensis Cameron, 1903:131.

Halictus manila Ashmead, 1904b:281.

Halictus luzonicus Strand, 1910:208.

Halictus javanensis Strand, 1910:198

Halictus amblypygus Strand, 1913

Halictus javanicus Friese, 1914:23. Bluthgen, 1926:492.

Lasioglossum (L) albescens (Smith); Michener, 1965: 173

Diagnosis: *Lasioglossum* (*Ctenomia*) *albescens* can be distinguished from other *Ctenomia* species by the following: size medium to large; body color grey-black; wing slightly cloudy grey; fine, wavy small ridges on the base of propodeum.

Coloration: body color grey-black because of the more pronounced shagreen; hair bands on tergites 2-5 rusty yellow; wing slightly cloudy grey to almost water-white, veins and spots brownish-yellow; flagella at ventral side sometimes red-brown to yellow-brown.

Structure: BL- 9.53mm

Head (Fig. 19): longer than wide. HW- 2.26mm HL- 2.06mm; EL- 1.58mm; CL- 0.5mm, CW- 0.6mm, IOD- 0.38mm clypeus complete black, shiny; paraoccular area sparsely covered by hairs.

Mesosoma (Fig. 20): smooth, silky matt with sparse; irregular arranged puncture on mesoscutum and scutellum; fine small longitudinal ridges or sometimes wavy long wrinkles on propodeal dorsum not reaching up to mid(Fig. 21); lateral Propodeal field smooth and shining with rather sparse punctures; Propodeal triangle usually smooth edged on the sides and top; tegula finely punctured; WL- 5.78mm.

Metasoma (Fig. 17): less shiny; elongate and oval; apical hair bands present T2- T5; silky, irregular spots on both sides at the base of the horizontal part of tergite 1, T1 dull with sparse punctures, T2 - T4 moderately punctuate; pygidial plate U-shaped; well developed scopa; basitibial plate oval, pointed apically; Inner hind tibial spur with 3 – 4 small teeth. (Fig. 22)

Flower record: Calendula, rose, cauliflower.

***Lasioglossum* (*Ctenomia*) *cavernifrons* Bluthgen, 1926 (Figs. 23-28)**

Halictus cavernifrons Bluthgen, 1926: 658

Diagnosis: *Lasioglossum* (*Ctenomia*) *cavernifrons* can be distinguished from other *Ctenomia* species by the following: size medium tolarge; body colour black; wing transparent; oblique ridges on the sides and less irregular, wrinkled stripes in middle on the base of propodeum.

Coloration: body colour shiny black; basal propodeum not carinate; hair bands on tergites T2-T4 white hair band; wing transparent; veins and spots brown; flagella ventrally reddish brown; tegula brown colored; hairs on the legs pale white.

Structure: BL- 8.41mm

Head (Fig. 25): Head almost as broad as thorax, as long as wide; HW- 2.21mm; HL- 2.10mm; EL- 1.58mm; CL- 0.61mm, CW- 0.59mm, IOD- 0.35mm clypeus complete black, shiny; area near clypeus with dense white hair; mandible upper jaw tip red; flagella ventrally reddish brown.

Mesosoma (Fig. 26): scutum and scutellum shiny, with extremely fine, flat dots, in the middle, distributed irregularly and more or less scattered; basal propodeum not carinate,less irregular oblique

Figs. 11-16 Female. *Halictus* (*Seladonia*) *propinquus*, 11- Dorsal habitus; 12- Lateral habitus; 13- Head; 14-Thorax; 15-Propodeum; 16-Hind tibial spur teeth

Figs. 17-22 Female. *Lasioglossum* (*Ctenomia*) *albescens*, 17-Dorsal habitus; 18- Lateral habitus; 19- Head; 20-Thorax; 21- Propodeum; 22- Hind tibial spur teeth

Figs. 23- 28 Female. *Lasioglossum* (*Ctenomia*) *cavernifrons*, 23-Dorsal habitus; 24- Lateral habitus; 25- Head; 26-Thorax; 27-Propodeum; 28-Hind tibial spur teeth

Figs. 29- 34 Female. *Lasioglossum* (*Ctenomia*) *sikkimense*, 29- Dorsal habitus; 30- Lateral habitus; 31- Head; 32-Thorax; 33- Propodeum; 34- Hind tibial spur teeth.

ridges on sides, wrinkled stripes in middle (Fig. 27); lateral propodeal field smooth and shining; tegula finely punctured; wings milky water-white, veins brown in colour; WL-5.91mm.

Metasoma (Fig. 23): is black brown, elongated egg-shaped, curved; tergum smooth, shining with very sparse and fine punctures; apical parts of legs more or less black, T1 not punctured,shiny; T2 and T3 with silky white hair band at the base with bands interrupted in middle; inner hind tibial spur with 3 teeth (Fig. 28).

Flower record: Lemon

***Lasioglossum* (*Ctenomia*) *sikkimense* (Blüthgen, 1926) (Figs. 29-34)**

Halictus sikkimensis Blüthgen, 1926: 586

Diagnosis: *Lasioglossum* (*Ctenomia*) *sikkimense* can be distinguished from other *Ctenomia* species by the following: size small; body brown black; Wingtransparent; reticulated ridges in middle on base of propodeum.

Coloration: body color brown black; hair bands on tergites T1-T3 pale white hair band; wing clear transparent; veins and spots yellowish brown; flagella ventrally reddish brown; tegula lightbrown colored; legs reddish brown; hairs on the legs pale white.

Structure: BL- 6.26mm

Head (Fig. 31): almost as long as wide; vertax flat; frons rough, densely punctured; HW- 1.65mm; HL- 1.62mm; EL- 1.11mm; CL- 0.39mm, CW- 0.49mm, IOD- 0.35mm clypeus complete black, punctured; paraoccular area, area near clypeus with white hair; mandible black with pre-apical tooth; flagella ventrally light brown.

Mesosoma (Fig. 32): scutum and scutellum not shiny, with irregular dense punctures; basal

propodeum with reticulated ridges in middle, edged on sides (Fig. 33); lateral Propodeal covered with hairs; tegula finely punctured; wings transparent, veins brown in color; WL- 4.11mm.

Metasoma (Fig. 29): chestnut brown, longer, elongated, oval shape; tergum smooth, shiningwith very sparse and fine punctures; apical parts of legs more or less black, T1 glossy, pale white lateral hair spot, T2 and T3 at the base with broad hair bands, T3 interrupted in middle; T4 and T5 with brown color hair bands; inner hind tibial spur with 4 teeth (Fig. 34).

Flower record: Cabbage, maize

***Lasioglossum* (*Ctenomia*) *splendidulum* (Vachal, 1895) (Figs. 35-40)**

Halictus splendidulus Vachal, 1895: 432

Halictus proteus Vachal, 1895: 438

Halictus semiaerinus Vachal, 1895: 443; Blüthgen, 1926: 611, 654

Halictus metenus Cockerell, 1937: 4; Ebmer, 1998: 376

Halictus (Evylaeus) bambusarum Cockerell, 1937: 10; Ebmer, 1998: 376

Halictus (Chloralictus) speculibasis Cockerell, 1937: 11; Ebmer, 1998: 376

Diagnosis: *Lasioglossum* (*Ctenomia*) *splendidulum* can be distinguished from other *Ctenomia* species by the following: size medium to large; body color black; finely punctuate; wings pale white; propodeun not carinate; less irregular oblique ridges reaching up to mid-length of basal propodeum.

Coloration: body color shiny brown black; T2-T5 white hair band; wings pale white; veins.

and spots dark brown; flagella ventrally dark brown; tegula chestnutbrown colored; hairs on the legs white.

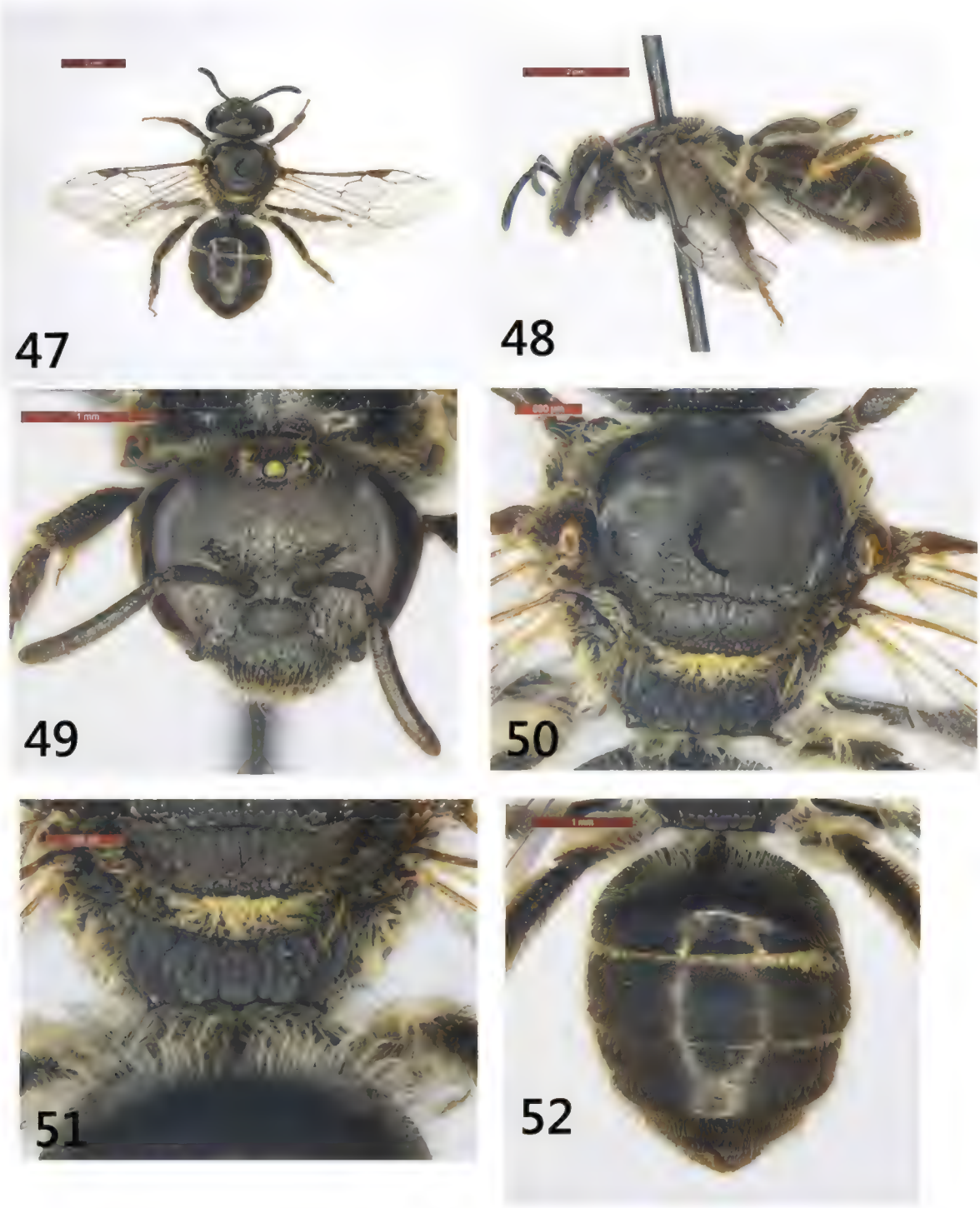
Structure: BL- 6.17mm

Head (Fig. 37): little wider than thorax; wider than long; HW- 1.62mm; HL- 1.46mm; EL- 1.06mm; CL- 0.27mm, CW- 0.41mm, IOD- 0.32mm clypeus brown black, shiny, sparsely punctured; area near clypeus with sparsely white hair; flagella ventrally brown.

Mesosoma (Fig. 38): scutum and scutellum shiny,

Figs. 35- 40 Female. *Lasioglossum* (*Ctenomia*) *splendidulum*, 35-Dorsal habitus; 36- Lateral habitus; 37- Head; 38-Thorax; 39-Propodeum; 40-Hind tibial spur teeth

Figs. 41- 46 Female. *Lasioglossum* (*Ctenomia*) *vagans*, 41-Dorsal habitus; 42- Lateral habitus; 43- Head; 44-Thorax; 45-Propodeum; 46- Hind tibial spur teeth



Figs. 47- 52 Female. *Patellapis* (*Pachyhalictus*) *liodoma*, 47- Dorsal habitus; 48- Lateral habitus; 49- Head; 50-Thorax; 51- Propodeum; 52-Abdomen

Figs. 53- 58 Female. *Patellapis* (*Pachyhalictus*) *reticulosa*, 53-Dorsal habitus; 54- Lateral habitus; 55- Head; 56-Thorax; 57-Propodeum; 58-Abdomen

with extremely fine, flat dots, in the middle distributed irregularly and more or less is scattered; basal propodeum with oblique ridges on the sides and less irregular in middle reaching up to mid length of basal propodeum (Fig. 39); lateral Propodeal field smooth and shining; tegula finely punctured; wings milky water-white, veins brown in color; WL- 5.91mm.

Metasoma (Fig. 35): black brown, elongated egg-shaped, curved; T1 smooth, shining with very sparse and fine punctures, T2,T3 and T4 with silky white hair band at the base with bands not interrupted in middle; apical parts of legs more or less black; inner hind tibial spur with 3 teeth (Fig. 40).

Flower record: Cabbage, maize

***Lasioglossum* (*Ctenomia*) *vagans* (Smith, 1857) (Figs. 41-46)**

Halictus vagans Smith, 1857: 42; Dalla Torre, 1896: 89; Blüthgen, 1931b: 327; Yasumatsu, 1935: 385; Baltazar, 1966: 367-368

Halictus cattulus Vachal, 1895: 437; Dalla Torre, 1896: 57; Blüthgen, 1926: 393; Blüthgen, 1926: 652, 670, 672; Blüthgen, 1930a: 72

Halictus cattulus var *peguanus* Vachal, 1895: 437; Blüthgen, 1926: 654

Halictus buddha Cameron, 1897: 107; Blüthgen, 1930a: 74

Halictus vishnu Cameron, 1897: 106; Blüthgen, 1930a: 74

Halictus phillipinensis Ashmead, 1904b: 128; Blüthgen, 1926: 416

Halictus matheranensis Cameron, 1907a: 1001; Blüthgen, 1930a: 77

Halictus emergendus Cameron, 1908a: 311; Blüthgen, 1926: 654

Halictus luteitarsellus Strand, 1910: 206; Blüthgen, 1926: 654

Halictus micado Strand, 1910: 204; Blüthgen, 1922: 54; Blüthgen, 1926: 386, 397

Halictus nasicensis Cockerell, 1911: 191; Blüthgen, 1926: 654

Halictus perhumilis Cockerell, 1911a: 192; Blüthgen, 1931b: 327

Halictus statialis Cockerell, 1911d: 667; Strand, 1913a: 29; Blüthgen, 1922: 63; Blüthgen, 1926: 386 [Notes]; Sonan, 1940: 375

Halictus bleharophorus Strand, 1913: 28; Blüthgen, 1923b: 242

Halictus centrophorus Strand, 1913c: 140; Blüthgen, 1926: 399

Halictus nalandicus Strand, 1913c: 140; Blüthgen, 1926: 399

Halictus javanicus Friese, 1914: 23

Halictus schmiedeknechti Friese, 1914: 24; Blüthgen, 1922: 56

Halictus phillipinensis var nigritarsellus Cockerell, 1919c: 274; Blüthgen, 1926: 407.

Halictus chaldaeorum Morice, 1921: 826; Blüthgen, 1922: 319; Cockerell, 1924a: 585; Blüthgen, 1926: 386

Halictus semivagans Cockerell, 1937: 5

Lasioglossum (*Ctenonomia*) *vagans* Pesenko, 1986: 121; Sakagami, 1989: 509; Ebmer, 1998: 377; Ebmer, 2004: 140

Diagnosis: *Lasioglossum* (*Ctenomia*) *vagans* can be distinguished from other *Ctenomia* species by the following: size small; body color brown black; sparsely punctuate; wings hyaline; propodeum carinate; irregular oblique ridges on basal propodeum.

Coloration: body color shiny black; metasoma chestnut brown; T2-T5 white hair band; wings hyaline; veins and spots brown; flagella ventrally brown; tegula light brown; legs yellow on tarsi;hairs on the legs white.

Structure: BL- 6-7mm

Head (Fig. 43): wider than long; finely punctured; HW- 1.77mm; HL- 1.53mm; EL- 1.20mm; CL- 0.31mm, CW- 0.50mm, IOD- 0.32mm clypeus brown black, shiny, punctured; paraoccular area, area near clypeus with sparsely white hair; flagella ventrally brown; mandible reddish brown apically.

Mesosoma (Fig. 44): scutum and scutellum not much shiny, with extremely fine, uniformly and more or less is scattered punctation; basal propodeum with irregular oblique ridges on basal propodeum (Fig. 45); lateral Propodeal field smooth and shining; tegula finely punctured; wings hyaline, sometimes brownish tint; veins brown in color; WL-4.27mm.

Metasoma (Fig. 41): is chestnut brown, elongated oval shaped; T1 glabrous, shining with fine punctures, T2-T5with white hair band at the base, T2 band interrupted in middle; apical parts of legs yellow; inner hind tibial spur with 3-4 teeth or 3 long teeth (Fig. 46).

Flower record: Cabbage, maize

Genus – *Patellapis*

***Patellapis* (*Pachyhalictus*) *liodoma* (Vachal, 1895) (Figs. 47-52)**

Halictus liodomus Vachal, 1895: 435

Pachyhalictus (*Pachyhalictus*) *liodomus* (Vachal, 1895); Michener, 1978: 518

Halictus scopipes Friese, 1918

Diagnosis: *Patellapis* (*Pachyhalictus*) *liodoma* can be distinguished from other *Pachyhalictus* species by the following: pronotum protruding side corners; scutum with net structure in middle, smooth on sides; mesonotum with central strong furrow.

Coloration: body color matt black; pubescence pale yellow; wings hyaline; veins light brown; flagella ventrally dark brown; tegula brown ; legs dark brown,light brown on tarsi; hairs on the legs yellow; clypeus brown black; eyes dark brown; mandible dark brown apically.

Structure: BL- 8-9mm

Head (Fig. 49): wider than long; rough; HW- 2.21 mm; HL- 1.83mm; EL- 1.40mm; CL- 0.40mm, CW- 0.75mm, IOD- 0.33mm, rough; paraoccular area, frons, finely reticulated; area near clypeus with sparsely white hair; frontal carina present.

Mesosoma (Fig. 50): pronotum projected side corners dorso-laterally; scutum with net structure in middle, smooth on sides; mesonotum with central strong furrow; basal propodeum with less reticulated sculpture, ridges with carina (Fig. 51).

Metasoma (Fig. 52): oval shaped; Hind basitibial plate pointed apically; T1 impunctate or sparsely punctuate in middle, glabrous, T2- T5 strongly punctuate, T2 punctate.

Patellapis (Pachyhalictus) reticulosa (Dalla Torre, 1896) (Figs. 53-58)

Halictus reticulatus_homonym Vachal, 1895.

Halictus reticulosus Dalla Torre, 1896:80.

Pachyhalictus (Pachyhalictus) reticulosa (Dalla Torre, 1896);

Michener, 1978: 518; Pesenko & Wu, 1997:288; Michener, 2000:370

Diagnosis: *Patellapis* (*Pachyhalictus*) *reticulosa* can be distinguished from other *Pachyhalictus* species by the following: size medium to large; body color matt black; rough head and thorax regulate; wings hyaline; propodeun carinate; strongly reticulate on basal propodeum.

Coloration: body color matt black; pubescence pale yellow;T2-T5 yellow hair band; wings hyaline; veins and spots dark brown to black; flagella ventrally brown; tegula brown ; legs brown on tarsi; hairs on the legs yellow.

Structure: BL-6.81mm.

Head (Fig. 55): as long as wide with tiny dense reticulation; HW-2.11mm;HL-2.10mm;EL-1.35mm;CL-1.26 mm; CW- 0.70mm; IOD-1.23mm;surface of supraclypeal area extensively reticulate; clypeus lack, punctured; area near clypeus with sparsely yellow hair; flagella ventrally black; mandible brown.

Mesosoma (Fig. 56): scutum extensively, irregular reticulation; tegula smooth, posteriorly impunctate; metanotum with dense pubescence basal propodeum shiny with strong, wider reticulate ridges;wings hyaline; veins dark brown in color; WL- 4.12mm.

Metasoma (Fig. 58): short, cylindrical; T1 glabrous at middle, T2 to T4 with lateral basal hair band interrupted medially; hind femur with long,

branched, pale and ventrally curved hairs; apical parts of legs more or less black.

Flower record: Dianthus and maize

ACKNOWLEDGMENTS

The authors are thankful to the Department of Biotechnology, Government of India, New Delhi 110003 for financial support. Authors also acknowledge Director, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India and Head, Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India, for all the facilities required for the study.

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(Received August 30, 2022; revised ms accepted November 11, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.794
ENTOMON 47(4): 415-420 (2022)
Short communication No. ent. 47407

Larvicidal effects of *Calotropis procera* leaf extracts against *Aedes aegypti* (L), vector of dengue fever

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ABSTRACT: Leaf extracts of *Calotropis procera* were tested against late third instar larvae of *Aedes aegypti* mosquito. Soxhlet extraction of the dried leaves powder with polar and non polar solvents (water, ethanol, hexane and acetone) was carried out. Larvicidal effects of plant extracts were observed after 24h of exposure. The control group showed no mortality. Ethanolic extract was found more toxic with LC_{50} 1.923 ppm and LC_{90} 8.83 ppm followed by aqueous extract (LC_{50} 2.607 ppm and LC_{90} 11.903 ppm), acetone extract (LC_{50} 4.1 ppm and LC_{90} 16.471 ppm) and hexane extract (LC_{50} 5.364 ppm and LC_{90} 31.759 ppm). As the ethanolic extract of *C. procera* leaves showed significant larvicidal properties, it can be used as an eco-friendly alternative for the control of *Ae. aegypti* vector. © 2022 Association for Advancement of Entomology

KEY WORDS: Ethanolic extract, probit analysis, toxicity, biopesticide

Mosquitoes transmit a myriad of harmful diseases like dengue, malaria, chikungunya, lymphatic filariasis and Japanese encephalitis. Approximately 700 million people suffer from such mosquito borne diseases each year that gradually results in about 1 million deaths annually (Taubes, 1997). The distribution of vector borne diseases is determined by complex demographic factors including environmental and social factors as well. Annual dengue incidences are estimated to be in the order of 100 million symptomatic and 300 million asymptomatic. The greatest burden is seen in Asia (75%) followed by Latin America (14%) and Africa. India suffers from three vector-borne diseases, malaria, lymphatic filariasis and visceral leishmaniasis (WHO, 2017). *Aedes aegypti* (Diptera, Culicidae) is the main vector of dengue and chikungunya (WHO, 2022). To control the proliferation of vector species of mosquitoes so many synthetic insecticides have been used worldwide. However, none of the formulations are promising due to its high cost, less environmental friendly, harmful effect on public health and increasing incidence of insecticide resistance. Because of these harmful effects on the public health and environment, herbal eco friendly formulations are in demand (Nerio *et al.,* 2010; Sritabutra *et al.,* 2011 and Reegan *et al.,* 2013). Further, as an alternative, the chemicals derived from the different parts of the plants can be used as a repellent, larvicide, ovipositional attractant and insect growth regulator (Babu and Murugan, 1998; Demirak and Canpolat, 2022).

Calotropis procera (Aiton) Dryand belongs to the family Asclepiadaceae and is mostly found in

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Bangladesh, India, Burma, Pakistan and the Sub-Himalayan tract. Indian traditional system of medicine, various parts of the plant are used for the treatment of various diseases like tumors, liver and abdomen diseases, piles, leprosy (CSIR, 1992; Kritikar and Basu, 1999). Moursy (1997) indicated its insecticidal and Markouk *et al.* (2000) larvicidal properties with their various solvents. Considering, the existing preliminary research (Sivagnaname and Kalyanasundaram, 2004; Thomas *et al.,* 2004; Cetin *et al.,* 2004; Ahmed and Hamshary, 2005; Shaleen *et al.,* 2005; Sharma *et al.,* 2006), the present study was focused on the potential of various solvent extracts of *C. procera* leaves against *Ae. aegypti* larvae.

Fresh leaves of *C. procera* were collected and washed with tap water and shaded dried at room temperature at 27±2°C for 15 days. Dried leaves were powdered with the help of an electrical grinder and then 30 g of the powder was extracted with 250 ml of polar and non polar solvents (water, ethanol, hexane and acetone) for 8 h using Soxhlet apparatus with boiling point ranging from 60–80°C followed by filtration through a Buchner funnel with Whatman number 1 filter paper (Vogel, 1978). The crude leaf materials were evaporated in a rotary vacuum evaporator. For the preparation of one per cent stock solution, one gram residue was taken and dissolved in 100 ml of solvent (same solvent that was used in the extraction process). Finally, concentrations ranging from 0.25 ppm to 20 ppm were used to carry out the experiments.

The larvae of *Ae. aegypti* were reared and colonized continuously in the National Centre for Disease Control laboratory. The temperature was kept 27 ± 2°C and maintained the humidity at 45 ±10 per cent and photoperiod 12:12 (light: dark). Larvae were kept in a water tray and the water was cleaned or changed every day to avoid toxic scum formation. Larvae were fed on yeast tablets. Late 3rd instar female larvae were kept in cages (30×30×30 cm) till the pupae were converted into adult mosquitoes. The adult mosquitoes were fed by rabbit blood meal and male mosquito was fed with 2 per cent glucose solution.

WHO (2005) guidelines were used to evaluate the larvicidal activity of extract of *C. procera*. Twenty-five late third instar larvae of *Ae. aegypti* were collected from the larval rearing bowl and moved in a 500 ml glass beaker (having 249 ml dechlorinated water and one ml of desired concentrations). Five replicates of each concentration and two replicates of controls were tested for each dilution under the laboratory conditions (ambient temperature 27 ±1°C and RH 75 – 80%). The control was prepared with 249 ml dechlorinated water and one ml of individual solvent. Larvae were exposed in dechlorinated water only (without solvent) prepared as a control. The larval percentage mortality was recorded for each test and controls after 24 h. LC_{50}, LC_{90} and other statistics like limits of upper and lower confidence limit (UCL and LCL) at 95 per cent confidence and chi-square values were calculated by probit analysis (Finney, 1971) and SPSS 16.0 version was used to find out the regression analysis.

In the larvicidal toxicity effects of *C. procera* leaves at various concentrations in different solvents against the dengue vector, *Ae. aegypti*, ethanol extracts showed the highest mortality rate with LC_{50} and LC_{90} values corresponding to 1.923 and 8.83 ppm respectively, followed by aqueous (LC_{50} and LC_{90} values 2.607 and 11.903 ppm respectively), acetone (LC_{50} and LC_{90} values 4.1 and 16.471ppm respectively), hexane (LC_{50} and LC_{90} values 5.364 and 31.759 ppm) respectively (Table 1). The larval mortality rate of *Ae. aegypti* increased with the increase in concentration of extracts. Ethanol extract of leaves of *C. procera* was found to be the most effective as compared to the other solvent extracts (Figs. 1, 2, 3 and 4).

The study established the usefulness of ethanolic leaf extract of *C. procera* plant against the late third or early forth instar larvae of *Ae. aegypti,* with LC_{50} and LC_{90} values at 1.923 and 8.83 ppm respectively, which shows relevance with the study conducted by Ramos *et al.* (2006) and Jazem *et al.* (2014) indicated medicinal properties of *C. procera* (leaves, roots and bark) against *Ae. aegypti*. Singh *et al.* (2005) showed the moderate larvicidal activity of the latex of *C. procera* against *Ae. aegypti, Anopheles stephensi*

Table 1. Larval toxicity of different solvents of *Calotropis procera* leaves against *Aedes aegypti*

Solvents	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	95% confidence limit LCL LC50 (LC90) UCL LC50 (LC90)		χ^2
Water	2.607	11.903	Y=1.943X-0.809	2.15(8.83)	3.14(18.17)	10.20*
Ethanol	1.923	8.83	Y=1.936X-0.549	1.56(6.58)	2.33(13.39)	8.49*
Acetone	4.1	16.471	Y=2.122X-1.3	3.49(13.27)	4.74(21.87)	8.19*
Hexane	5.364	31.759	Y=1.659X-1.21	4.52(24.35)	6.27(45.30)	21.92*

*Control – nil mortality; within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT; LCL - lower confidence limit, UCL - upper confidence limit, *P<0.05 level*

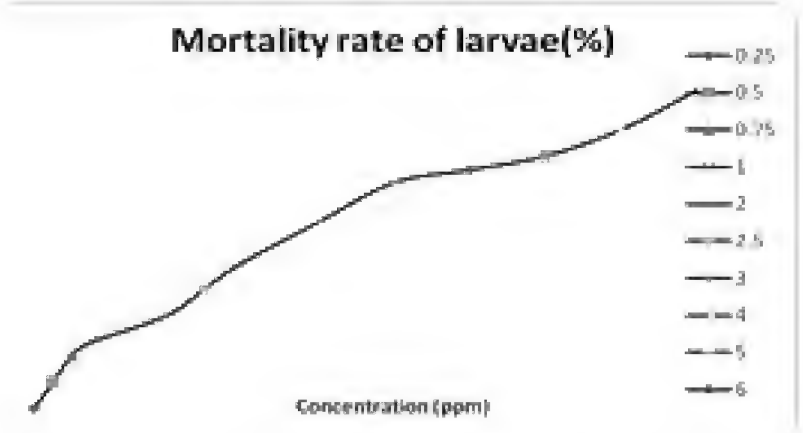

Fig. 1 Toxicity of aqueous extract of *Calotropis procera* against *Ae. aegypti*

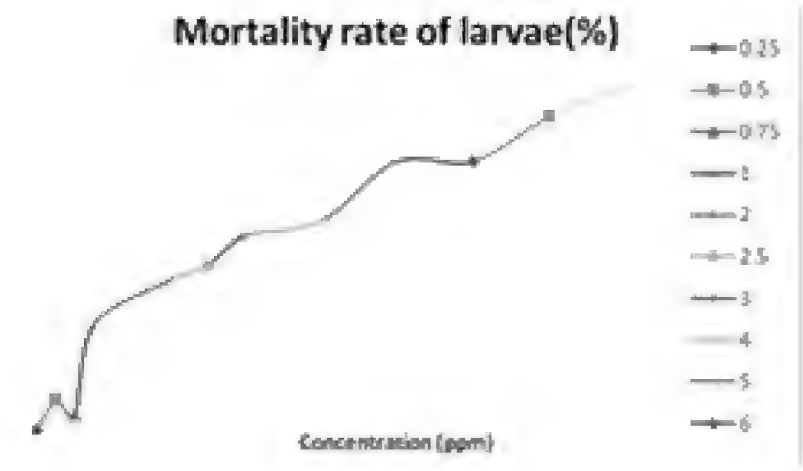

Fig. 2 Larval toxicity of ethanol extract of *Calotropis procera* against *Ae. aegypti*

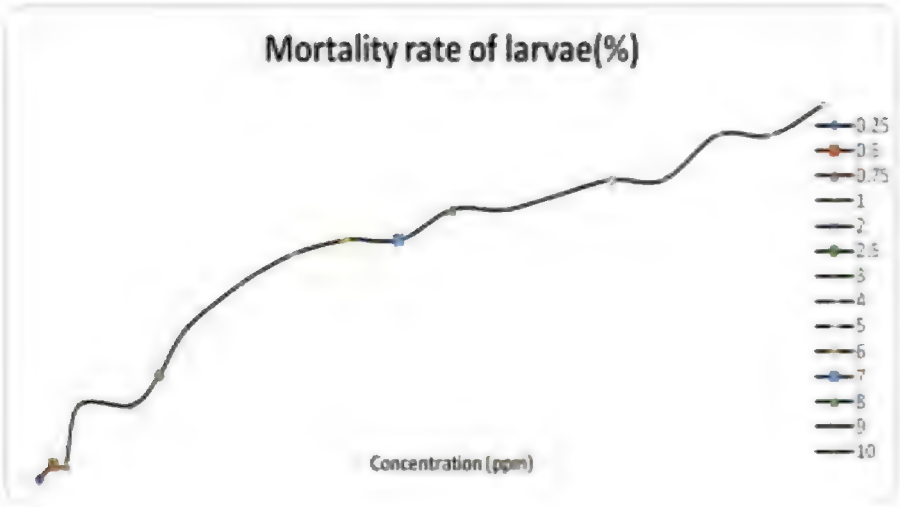

Fig. 3 Larval toxicity of acetone extract of *Calotropis procera* against *Ae. aegypti*

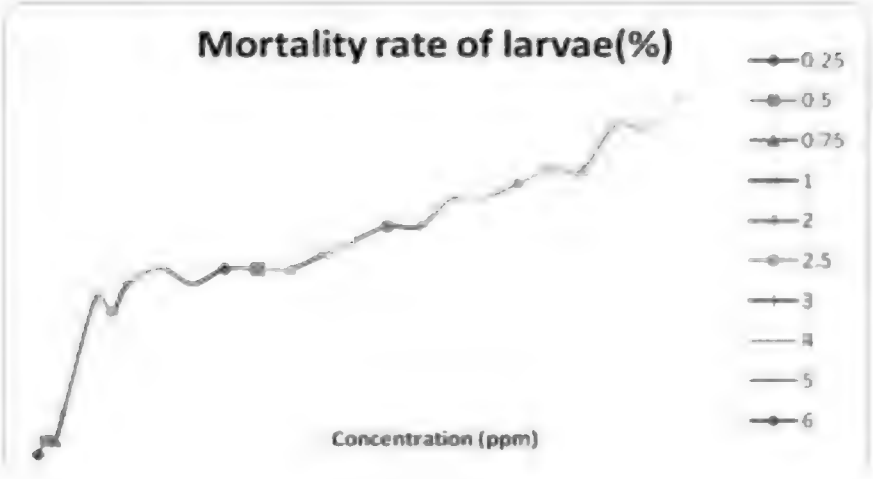

Fig. 4 Larval toxicity of hexane extract of *Calotropis procera* against *Ae. aegypti*

and *Culex quinquefasciatus*. Shreya *et al.* (2012) concluded the LD_{50} value of the ethanolic leaves extract of *Calotropis* spp. against *Ae. aegypti* as 351.43 (95% CI: 345.64-345.51) which shows the resemblance with the present study. The toxicity of different parts of the *C. procera* plant has also been reported earlier against mosquitoes by Staples and Herbst in 2005. *Calotropis* plant has been in use for the prevention of so many diseases for a long time due to its medicinal properties (Dewan, 2000; Van *et al.,* 2005; Chitme *et al.*, 2005; Argal and Pathak, 2006). Application of 3 ml *C. procera* leaves extract per 100 ml solvent recorded 100 percent mortality against *Ae. aegypti* (Singh *et al.*, 2005).

Yakubu *et al.* (2021) reported LC_{50} of *C. procera* leaves extract against *Ae. aegypti* and *Cx. quinquefasciatus* at 0.116mg/ml and 0.249mg/ml respectively. The present study indicates that the leaves of *C. procera* have larvicidal properties against dengue vector *Ae. Aegypti.* As *C. procera* is an easily available medicinal plant, its phytochemicals may be less expensive and relatively safe for environment. Hence the ethanolic extract of *C. procera* leaves could be an effective

alternative to synthetic insecticides for the control of *Ae. Aegypti.*

ACKNOWLEDGEMENTS

Authors are thankful to the Lovely Professional University, Punjab, India for providing all support and National Centre for Disease Control, Delhi, for providing laboratory and insectary facilities for the present work.

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(Received August 08, 2022; revised ms accepted November 01, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.795
ENTOMON 47(4): 421-424 (2022)
Short communication No. ent. 47408

Altitude specific leaf quality of the host plants of tasar silkworm *Antheraea mylitta* Drury (Lepidoptera, Saturniidae) in Similipal Biosphere Reserve, Odisha, India

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ABSTRACT: Altitudinal variation and role of leaf nutrients in the host plants of tasar silkworm *Antheraea mylitta* Drury (Lepidoptera, Saturniidae) influences, the rearing, grainage and quantitative traits of tasar and in the quality of cocoon formed. The present works analysed the nutritional status of the tasar host plant leaves of asan (*Terminalia tomentosa*) and arjun (*T. arjuna*) collected from Kendujuani (508 m ASL), Mudrajodi (223 m ASL) and Kuliana (64 m ASL) in the district of Mayurbhanj, in Similipal Biosphere Reserve, Odisha. The study revealed that, nutritional value of asan leaves is better at a higher altitude (Kendujuani). The concentration of ascorbic acid in the leaves of asan and arjun was found higher in the leaves from Kendujuani.

KEYWORDS: *Terminalia tomentosa*, *T. arjuna*, ascorbic acid, chlorophyll, phenolics, protein

Tropical tasar silkworm a wild type *Antheraea mylitta* Drury (Lepidoptera, Saturniidae) is polyphagous in nature and reared outdoor on arjun (*Terminalia arjuna*) and asan (*T. tomentosa*). Similipal Biosphere Reserve (SBR) is situated in Mayurbhanj district of Odisha in India between 21°28'-22°8' north latitude and 86°4'-86°37' east longitude. Mayurbhanj is the largest tasar producing district in Odisha. The wild ecoraces are mainly distributed in high altitude of SBR and all are mostly univoltine in nature (Singh and Srivastava,1997; Dey *et al.*, 2010). The thickness of leaf increases with enhancing altitude (Körner, 2003; Zhang *et al.*, 2014). Although there are reports on rearing behaviour on different food plants, there is scanty information on the basis of altitudinal variation and role of leaf nutrients in controlling the rearing, grainage and quantitative traits of tasar silkworm *A. mylitta* along with effect on the nutritional status of some biomolecules of the arjun and asan. The study was conducted in three sericulture farms, viz., Kendujuani (508 m ASL), Mudrajodi (223 m ASL) and Kuliana (64 m ASL) in the district of Mayurbhanj, Odisha, India during the rearing period of tasar silkworm on primary host plant leaves. In the present study various biochemical constituents of host leaves like protein, ascorbic acid, total carbohydrate, total phenolic and total chlorophyll content of the host leaves of different eco-pockets of SBR on the basis of altitude analysed.

Collection of leaf samples: In all the experiments freshly green leaves of asan and arjun plant were collected from the above-mentioned farms. Samples

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were placed in clean polyethylene bags, sealed and transported under refrigerated condition to the laboratory. For further analyses samples were washed under running tap water to remove the adhering dirt and stored at -20°C. Analysis was completed within 24 hours of sample collection.

Biochemical Analysis: Five grams of each leaf samples were homogenized in ice-cold extraction buffer. The homogenates were centrifuged for 20 min at 10,000 rpm. The supernatants were collected for further biochemical analyses. Protein concentrations of various samples were estimated by the method of Lowry *et al.* (1951). Ascorbic acid concentration was measured according to the method of Jagota and Dani (1982).Carbohydrate concentration was measured according to the method of Yemm and Willis (1954). Total phenolic content was measured according to the method of Slinkard and Singleton (1977). Total chlorophyll content was measured according to the method of Anderson and Boardman (1964).

Statistical analysis was performed for mean values and standard deviation, besides analysis of variance. Differences were considered statistically significant when $p < 0.05$. Tukey's post-hoc test was done to establish the honest significant difference (HSD) or Critical Difference (CD) among the mean values (Tukey, 1977). All the analyses were carried out by using MS-Excel software package and Statistics.

Leaf biochemical contents of asan: Total protein content of leaf tissues of asan at Kendujuani showed the higher value than that at the Mudrajodi and Kuliana. Ascorbic acid concentration of leaf tissues from Mudrajodi and Kuliana was lower than that of Kendujuani. In the case of total carbohydrate concentration both at Kendujuani and Mudrajodi were at par with each other and higher values over Kuliana. The level of total leaf phenol content at Kuliana and Mudrajodi was higher than that found at the Kendujuani. Total chlorophyll concentration at all three places were at par with each other (Table 1).

Leaf biochemical contents of arjun: Highest level of total protein was found at Kendujuani. The ascorbic acid content in all three places was at par with each other. Total carbohydrate concentration both at Kendujuani and Mudrajodi were at par with each other and were higher values over Kuliana. Reverse pattern was found in the case of total phenol, i.e., Kuliana and Mudrajodi had almost similar values with lower value at Kendujuani. The concentration of total chlorophyll was highest at Kendujuani and slightly lower at Mudrajodi, while lowest at Kuliana (Table 2).

Deka and Kumari (2013) corroborates with the findings, that leaf proteins have an important role for production of silk. The leaves enhanced with protein showed significance on production of cocoon. Tasar silkworm, *A. mylitta* has tremendous ability to convert the leaf proteins into synthesis of silk with the silk gland. Kendujuani is placed at medium altitude suitable for Daba variety indicates a better source of protein for the larva of *A. mylitta*, as dietary proteins provided essential amino acids needed for building of new tissues. All type of proteins present in the host plant leaves are digested and assimilated in silkworm gut and converted into body matter and also silk filaments leading to formation of cocoon (Krishnaswami, 1978). Ascorbic acid acts as a catalyst in redox reactions which has the strong ability to reduce the reactive oxygen species (ROS) (Padayatty *et al.*, 2003). In addition to its antioxidant potentials, ascorbate also acts as substrate for ascorbate peroxidase, the redox enzyme which has a strong role in stress resistance function of plants (Shigeoka *et al.*, 2002). High ascorbic acid concentration in the Asan and Arjun leaves at Kendujuani (Table 1, 2) corroborates the findings of Shigeoka *et al.* (2002). It may be suggested that this host plant ascorbic acid content providing stress resistance and also fighting extremities of climatological factors, like temperature, relative humidity etc. Our results also demonstrate high carbohydrate concentration of food at Kendujuani while lowest at Kuliana, indicating high carbohydrate content of food found to be gaining in larval mass as reported earlier (Bernays and Chapman, 1994). Deka and Kumari (2013) ascribed higher carbohydrate content of asan leaf to the higher rate of photosynthesis. Carbohydrates are required for the energy metabolism too. In the plants phenols have the

Table 1. Tukey's post-hoc test on the quality of Asan and Arjun leaf tissue (n=10)

Ecopocket	Protein (mg g^{-1})	Ascorbic acid (µg g^{-1})	Carbohydrate (mg g^{-1})	Phenolics (mg g^{-1})	Chlorophyll (mg g^{-1})
Asan leaf tissue					
Kendujuani	257.0^{a}	1.58^{a}	2.91^{a}	30.97^{b}	3.17^{a}
Mudrajodi	225.0^{b}	1.46^{b}	2.71^{a}	32.58^{a}	2.50^{b}
Kuliana	218.0^{b}	1.34^{b}	2.08^{b}	33.66^{a}	2.45^{b}
CD	12.7^{***}	0.12^{***}	0.39^{***}	1.52^{ns}	0.54^{**}
Arjun leaf tissue					
Kendujuani	219.29^{a}	1.51^{a}	4.45^{a}	31.68^{b}	3.09^{a}
Mudrajodi	209.26^{b}	1.27^{b}	3.71^{b}	32.08^{b}	2.81^{b}
Kuliana	206.51^{b}	1.23^{b}	1.96^{c}	34.42^{a}	2.60^{b}
CD	7.77^{*}	0.21^{*}	0.62^{***}	2.01^{ns}	0.22^{**}

Note: The superscripts a, b and c denote the grouping of parameter values based on Tukey's Post hoc test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ^{ns} *– Not significant (ANOVA)*

functions like defense against pests and diseases, herbivores, phytophagous insects and fungal, bacterial pathogens (Lappartient and Touraine, 1997; Strack, 1997; Jones and Hartley, 1999; Lappartient *et al.*, 1999; Wuyts *et al.*, 2006). In the present study, the level of total leaf phenol content in Kuliana and Mudrajodi was significantly higher than that of the Kendujuani (Table 1, 2), that supports the findings of Sawa *et al.* (1999) that phenols have the role of antioxidants with free radical scavenging capacity, where they break the free radical chain reaction by donating hydrogen atom. In many plants phenolic compounds found to be protect leaves from photo damage. In our present investigation total chlorophyll concentration in all three places were found to be identical, the variation indicates that the chlorophyll content of primary food plants plays a pivotal role for the successful larval rearing resulting to higher cocoons as well as better quality of silk for commercial purpose as reported by Baskey *et al.* (2019). According to Sujathamma and Dandin (2000) the higher chlorophyll content in mulberry leaves adjudicates the higher photosynthesis rate, thus it serves as one of the important criteria in evaluating leaf quality.

Considering overall performance of host plants nutritional status, and *A. mylitta* rearing behaviour it was revealed that the Kendujuani is the most conducive site for tasar silkworm rearing followed by Mudrajodi and Kuliana. Mudrajodi shows the moderate trend so, in order to achieve targeted productivity of tasar cocoons with good silk content, nutrient management in the plant needed to be adopted properly. The leaf parameters at different altitudes may have some effect on leaf nutrition, i.e., leaves may have different nutritional status at different places. So, nutrient management is required at lower altitude, i.e., Kuliana for gainful tasar cultivation.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to Head of the Department, P.G. Department of Zoology, MSCBDU for providing necessary laboratory facilities. Also thankful to all the staff members of Regional Sericultural Research Station, Central Silk Board, Baripada, TRCS, Kendujuani, Mudrajodi, Kuliana and Assistant Director of Sericulture, Baripada, Govt. of Odisha for their immense help and cooperation in sample collection.

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(Received April 19, 2022; revised ms accepted September 02, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.796
ENTOMON 47(4): 425-432 (2022)
Short communication No. ent. 47409

A checklist of Erebinae (Lepidoptera, Erebidae) from India

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ABSTRACT: Species under the subfamily Erebinae in India has been compiled and updated. A total of 250 species under 65 genera is enumerated. Current systematic status of the species based on the molecular phylogenetic studies by Zahiri *et al.* (2011) is given along with their type species and generic synonyms. © 2022 Association for Advancement of Entomology

KEY WORDS: Morphology, redescription, variation, Western Ghats, distribution species, genera, synonyms, systematic status

Family Erebidae, one of the diverse families of moths of superfamily Noctuoidea comprises about 25000 described species all over the world (Van Nieukerken *et al.*, 2011). Erebinae, a major subfamily of the family Erebidae of the superfamily Noctuoidea, consists of more than 10,000 described species (Singh and Ranjan, 2016; Zahiri *et al.*, 2011). Erebinae has a very complex taxonomic history. Fibiger and Lafontain (2005) divided Noctuoidea into nine families including Erebidae, and redefined Noctuoidea including five families namely Oenosandridae, Doidae, Notodontidae, Micronoctuidae and Noctuidae under it. All quadrifid groups including Erebinae were shifted to the family Noctuidae. The current taxonomic status of Erebinae is based on the molecular phylogenetic studies by Zahiri *et al.* (2011).

Data regarding species of the subfamily Erebinae from India is remain scattered in literature. Many genera of the Erebinae subfamily are placed under outdated classification (Homziak *et al.*, 2016). In 1894 Hampson recorded many Erebinae species in his book 'Fauna of British India: Moths' (volume 2 and 3) under the subfamilies Quadrifinae and Focillinae of Noctuidae. In a study on the moth fauna of Orissa, Mandal and Maulik (1991) reported several species of Erebinae belonging to the genera *Lagoptera* Guenée, *Speiredonia* Hubner, *Anua* Walker, *Parallelia* Hubner and *Chalciope* Hubner. However, many of these genera are not valid now. Genus *Lagoptera* is considered as a synonym of *Thyas* Hübner (Poole, 1989). Similarly, species of the genus *Anua* were shifted to the genus *Ophiusa* Ochsenheimer (Poole, 1989). Smetacek (2008) recorded 887 species at different elevations of Nainital district (Utharkhand, India) mainly from Kummon (Himalaya). *Bastilla maturescens* Walker, *B. praetermissa* William Warren and *B. analis* (Guenee) reported by Smetacek (2008) are now considered as synonyms of species of *Dysgonia* Hubner (Poole, 1989). Gadhikar *et al.* (2015), Paul *et al.* (2017), Gurule (2013) and Sondhi and Sondhi (2016) also reported *Bastilla* Swinhoe moths from India. Some of the moth species of *Bastilla* are now shifted to the genus *Dysgonia* while some are retained in the *Bastilla* genus itself. Genus *Caranilla* Moore and *Pindara* Fabricius are also synonymized to *Dysgonia* by Poole 1989. *Caranilla* and *Pindara* species reported by Rose

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(2002) and Sivasankaran *et al.* (2017) is now treated under the genus *Dysgoina*.

In this context, a list of moths of subfamily Erebinae reported so far from India is compiled and enumerated. The current systematic status of the species based on the molecular phylogenetic studies by Zahiri *et al.* (2011) is given along with their type species and generic synonyms.

Systematic List

Order **Lepidoptera**

Superfamily **Noctuoidea**

Family **Erebidae**

Subfamily **Erebinae**

Checklist of Erebinae (Lepidoptera, Erebidae) from India

Genus ***Ischyja*** Hübner

Type species *Ischyja manlia* Cramer

1. *I. inferna* Swinhoe
2. *I. manlia* Cramer
3. *I. ferrifracta* Walker
4. *I. marapok* Holloway
5. *I. schlegelii* Snellen
6. *I. hagenii* Snellen
7. *I. hemiphaea* Cramer

Genus ***Ophisma*** Guenée

Type species *Ophisma gravata* Guenée

8. *O. pallescens* Walker
9. *O. gravata* Guenée

Genus ***Serrodes*** Guenée

Type species *Serrodes inara* Cramer

10. *S. campana* Guenée
11. *S. mediopallens* Prout
12. *S. inara* Cramer
13. *S. caesia* Warren

Genus ***Grammodes*** Guenée

Type species *Grammodes geometrica* Fabricius

Synonym: *Prodotis* John

14. *G. stolida* Fabricius
15. *G. geometrica* Fabricius

Genus ***Mocis*** Hübner

Type species *Phalaena virbia* Cramer

Synonyms: *Remigia* Guenée, *Pelomi* Warren, *Baratha* Walker

16. *M. frugalis* Fabricius
17. *M. undata* Fabricius
18. *M. discios* Kollar
19. *M. laxa* Walker

Genus ***Ophiusa*** Ochsenheimer

Type species *Phalaena tirhaca* Cramer

Synonyms: *Anua* Walker, Hemachra Sodoffsky, Meropis Hübner, Peranua Berio, Perophiusa Berio, Trichanua Berio.

20. *O. olista* Swinhoe
21. *O. triphaenoides* Walker
22. *O. trapezium* Guenée
23. *O. discriminans* Walker
24. *O. disjungens* Walker
25. *O. indistincta* Moore
26. *O. tirhaca* Cramer
27. *O. mcjanesi* Guenée
28. *O. crameri* Moore
29. *O. pseudotirhaca* Singh & Ranjan

Genus ***Erebus*** Latreille

Type species *Phalaena crepuscularis* Linnaeus

Synonyms: *Argiva* Hübner, *Bocana* Walker, *Byas* Billberg, *Cariona* Swinhoe, *Eupatula* Ragonot, *Patula* Guenée

30. *E. macrops* Linnaeus
31. *E. ephesperis* Hübner
32. *E. caprimulgus* Fabricius
33. *E. hieroglyphica* Drury
34. *E. crepuscularis* Linnaeus
35. *E. albicinctus* Kollar
36. *E. strigipennis* Moore
37. *E. gemmans* Guenée
38. *E. glaucopis* Walker

39. *E. jaintiana* Swinhoe

Genus ***Lygniodes*** Guenée
Type species *Agonista hypoleuca* Guen
Synonyms: *Agonista* Rogenhofer
40. *L. schoenbergi* Pagenstcher
41. *L. hypoleuca* Guenée
42. *L. ciliata* Moore
43. *L. vampyrus* Fabricius

Genus ***Ulotrichopus*** Wallengren
Type species *Ulotrichopus tortuosus* Wallengren
Synonyms: *Alura* Möschler
44. *U. macula* Hampson

Genus ***Avatha*** Walker
Type species *Avatha includens* Walker
Synonyms: *Pseudathyrma* Butler, *Pterochaeta* Holland
45. *A. noctuoides* Guenée
46. *A. bubo* Geyer
47. *A. chinensis* Warren
48. *A. discolor* Fabricius
49. *A. bipartite* Wileman

Genus ***Trigonodes*** Guenée
Type species *Phalaena hyppasia* Cramer
50. *T. hyppasia* Cramer
51. *T. disjuncta* Moore

Genus ***Ercheia*** Walker
Type species *Ercheia diversipennis* Walker
52. *E. cyllaria* Cramer
53. *E. diversipennis* Walker
54. *E. niveostrigata* Warren
55. *E. umbrosa* Butler

Genus ***Pandesma*** Guenée
Type species *Pandesma quenavadi* Guenée
Synonyms: *Cerbia* Walker, *Michera* Walker, *Subpandesma* Berio, *Thria* Walker, *Vapara* Moore
56. *P. quenavadi* Guenée
57. *P. robusta* Walker
58. *P. anysa* Guenée

Genus ***Lacera*** Guenée
Type species *Phalaena alope* Cramer
59. *L. noctilio* Fabricius
60. *L. alope* Cramer
61. *L. procellosa* Butler

Genus ***Ericeia*** Walker
Type species *Ericeia sobria* Walker
Synonyms: *Girpa* Walker, *Villosa* Koch, *Erceia* Turner
62. *E. inangulata* Guenée
63. *E. eriophora* Guenée
64. *E. korintijiensis* Prout
65. *E. pertendens* Walker

Genus ***Artena*** Walker
Type species *Artena submira* Walker
66. *A. inversa* Walker
67. *A. dotata* Fabricius
68. *A.submira* Walker

Genus ***Thyas*** Hübner
Type species *Thyas honesta* Hübner
Synonyms: *Lagoptera* Guenée, *Dermaleipa* Saalmüller
69. *T. coronata* Fabricius
70. *T. juno* Dalman
71. *T. honesta* Hübner

Genus ***Achaea*** Hübner
Type species *Phalaena melicerta* Drury
Synonyms: *Geria* Walker, *Heliophisma* Hampson
72. *A. janata* Linnaeus
73. *A. serva* Fabricius
74. *A. mezentia* Stoll
75. *A. mercatoria* Fabricius

Genus ***Spirama*** Guenée
Type species *Phalaena retorta* Clerck
Synonyms: *Spiramia* Walker
76. *S. retorta* Clerck
77. *S. helicina* Hübner
78. *S. unistrigata* Guenee

79. *S. triloba* Guenée
80. *S. indenta* Hampson
81. *S. vespertilio* Fabricius

Genus ***Hypopyra*** Guenée
Type species *Noctua vespertilio* Fabricius
Synonyms: *Emmonodia* Walker, *Maxula* Walker, *Pyramarista* Kirby
82. *H. vespertilio* Fabricius
83. *H. ossigera* Guenée
84. *H. unistrigata* Guenée

Genus ***Pericyma*** Herrich-Schäffer
Type species *Acidalia albidentaria* Freyer,
Synonyms: *Alamis* Guenée, *Homoptera* Walker, *Dugaria* Walker, *Moepa* Walker, *Ozopteryx* Saalmüller
85. *P. albidens* Walker
86. *P. glaucinans* Guenée
87. *P. cruegeri* Butler
88. *P. umbrina* Guenée

Genus ***Anisoneura*** Guenée
Type species *Anisoneura salebrosa* Guenée
89. *A. aluco* Fabricius
90. *A. hypocyanea* Guenée
91. *A. salebrosa* Guenée

Genus ***Dysgonia*** Huebner
Type species *Phalaena algira* Linnaeus
Synonyms: *Caranilla* Moore, *Pindara* Moore
92. *D. rogenhoferi* Bohatsch,
93. *D. crameri* Moore
94. *D. rigidistria* Guenée
95. *D. torrida* Guenee
96. *D. stuposa* Fabricius
97. *D. latifascia* Warren
98. *D. properata* Walker
99. *D. algira* Linnaeus
100. *D. illibata* Fabricius
101. *D.conficiens* Walker

Genus ***Bastilla*** Swinhoe
Type species *Ophiusa redunca* Swinhoe
Synonyms: *Naxia* Guenée, *Xiana* Nye
102. *B. conficiens* Walker
103. *B. maturata* Walker
104. *B. arctotaenia* Guenée
105. *B. fulvotaenia* Guenée
106. *B. acuta* Moore
107. *B. maturescens* Walker
108. *B. joviana* Stoll
109. *B. amygdalis* Moore
110. *B. absentimacula* Guenée
111. *B. praetermissa* Warren
112. *B. analis* Guenée
113. *B. angularis* Boisduval
114. *B. arcuata* Moore
115. *B. simillima* Guenée

Genus ***Avitta*** Walker
Type species *Avitta subsignans* Walker
Synonyms: *Asta* Walker, *Oroba* Walker
116. *A. rufifrons* Moore
117. *A. fasciosa* Moore
118. *A.quadrilinea* Walker
119. *A. subsignans* Walker

Genus ***Ommatophora*** Guenee
Type species *Phalaena luminosa* Cramer
120. *Ommatophora luminosa* Cramer

Genus ***Ascalapha*** Hübner
Type species *Phalaena odorata* Linnaeus
Synonyms: *Idechthis* Hübner, *Otosema* Hübner
121. *Ascalapha odorata* (Linnaeus

Genus ***Polydesma*** Boisduval
Type species *Polydesma umbricola* Boisduval
Synonyms: *Anodapha* Moore, *Anthemoessa* Agassiz, *Anthemoisia* Blanchard, *Trichopolydesma* Berio
122. *P. boarmoides* Guenée
123. *P. albicola* Walker
124. *P. turbata* Walker
125. *P. sublimis* Felder
126. *P. umbricola* Boisduval
127. *P. otiosa* Guenée
128. *P. praecedens* Walker

Genus ***Hulodes*** Guenée
Type species *Phalaena caranea* Cramer
Synonyms: *Hylodes* Hampson
129. *H. caranea* Cramer
130. *H. monostriata* Guenee
131. *H. drylla* Guenée

Genus ***Fodina*** Guenée
Type species *Fodina oriolus* Guenée
Synonyms: *Anocala* Scott
132. *F. cuneigera*
133. *F. stola* Guenée
134. *F. pallula* Guenée
135. *F. oriolus* Guenée

Genus ***Catocala*** Schrank
Type species *Phalaena nupta* Linnaeus
Synonyms: *Andreusia* Hampson, *Blepharidia* Hübner, *Divercala* Beck, *Hemigeometra* Haworth, *Koraia* Herz, *Promonia* Beck
136. *C. armandi* Poujade
137. *C. tapestrina* Moor
138. *C. dotatoides* Poole
139. *C. prolifica* Walker
140. *C. macula* Hampson
141. *C. patala* Felder & Rogenhofer
142. *C. nymphaea* Esper
143. *C. flavescens* Hampson
144. *C. distorta* Butler
145. *C. nupta* Linnaeus
146. *C. concubia* Walker
147. *C. nivea* Butler
148. *C. afghana* Swinhoe
149. *C. amnonfreidbergi* Kravchenko

Genus ***Macaldenia*** Moore
Type species *Hulodes palumba* Guenée
Synonyms: *Parallelura* Berio
150. *Macaldenia palumba* Guenée

Genus ***Entomogramma*** Guenée
Type species *Entomogramma fautrix* Guenée
Synonyms: *Taramina* Moore
151. *E. torsa* Guenée
152. *E. fautrix* Guenée
153. *E. mediocris* Walker

Genus ***Attatha*** Moore
Type species *Hypercompa regalis* Moore
Synonyms: *Arattath***a** Janse
155. *A. ino* Drury
156. *A. regalis* Moore

Genus ***Lyncestis*** Walker
Type species *Phalaena amphix* Cramer
Synonyms: *Jarasana* Moore
157. *Lyncestis amphix* Cramer

Genus ***Homaea*** Guenée
Type species *Homaea clathrum* Guenée
158. *Homaea clathrum* Guenée

Genus ***Sypnoide*****s** Hampson
Type species *Sypna mandarina* Leech
Synonyms: *Hyposypnoides* Berio, *Equatosypna* Berio, *Pysnoides* Berio, *Supersypnoides* Berio
159. *S. rubrifascia* Moore
160. *S. cyanivitta* Moore
161. *S. mandarina* Leech
162. *S. curvilinea* Moore
163. *S. kirbyi* Butler
164. *S. prunosa* Moore
165. *S. rectilinea* Moore

Genus ***Hypersypnoides*** Berio
Type species *Hypersypnoides congoensis* Berio
Synonyms: *Othresypna* Berio
166. *H. punctosa* Walker
167. *H. submarginata* Walker
168. *H. caliginosa* Walker
169. *H. catocaloides* Moore
170. *H. constellata* Moore
171. *H. marginalis* Hampson
172. *H. pulchra* Butler

Genus ***Daddala*** Walker

Type species *Daddala quadrisignata* Walker

Synonyms: *Elpia* Walker

173. *D. lucilla* Butler

174. *D. quadrisignata* Walker

175. *D. brevicauda* Wileman & South

Genus ***Erygia*** Guenée

Type species *Erygia apicalis* Guenée

Synonyms: *Calicula* Walker, *Erygansa* Bethune Baker, *Felinia* Guenée, *Ansa* Walker

176. *E. apicalis* Guenée

177. *E. spissa* Guenée

178. *E. reflectifascia* Hampson

Genus ***Acantholipes*** Lederer

Type species *Noctua regularis* Hübner

Synonyms: *Acantholipis* Hampson, *Docela* Walker, *Isatoolna* Nye, *Lasionota* Warren, *Nolaseniola* Strand

179. *A. pansalis* Walker

180. *A. trajecta* Walker

181. *A. lagusalis* Walker

182. *A. circumdata* Walker

183. *A. hypenoides* Moore

184. *A. similis* Moore

185. *A. miser* Butler

186. *A fasciosus* Moore

187. *A. gemma* Swinhoe

Genus ***Aedia*** Hübne

Type species *Noctua funesta* Esper

Synonyms: *Acanthodelta* Wiltshire, *Melanephia* Hampson, *Renatia* Berio, *Syagrana* Wiltshire

188. *A. acronyctiodes* Guenée

189. *A. leucomelas* Linnaeus

190. *A. squamosa* Wallengren

191. *A. perdicipennis* Moore

Genus ***Bamra*** Moore

Type species *Agriopis discalis* Moore

Synonyms: *Ostacronycta* Bethune-Baker

192. *B. albicola* Walker

193. *B. mundata* Walker

194. *B. lepida* Moore

Genus ***Chalciope*** Hübner

Type species *Chalciope mygdon* Cramer

Synonyms: *Euclidisema* Hampson

195. *Chalciope mygdon* Cramer

Genus ***Catephia*** Ochsenheimer

Type species *Noctua alchymista* Denis & Schiffermüller

Synonyms: *Nagia* Walker, *Anoplia* Stephens, *Mageutica* Hampson

196. *C. linteola* Guenée

197. *C.dentifera* Moore

198. *C. inquieta* Walker

199. *C. dulcistriga* Walker

200. *C. squamosa* Wallengren

201. *C. flavescens* Butler

Genus ***Buzara*** Walker

Type species *Buzara chrysomela* Walker

202. *B. umbrosa* Walker

203. *B. onelia* Guenée

Genus **Agassiz** Guenée

Type species *Phalaena chlorea* Cramer

Synonyms: *Sphingimorpha* Hacker

204. *S. chlorea* Cramer

Genus ***Anomis*** Hübner

Type species *Anomis erosa* Hübner

Synonyms: *Amarna* Walker, *Anomus* Agassiz, *Capitaria* Walker, *Cosmophila* Boisduval, *Gonotis* Moore, *Gonitis* Guenée

205. *A. figlina* Butler

206. *A. revocans* Walker

207. *A. lineosa* Walker

208. *A. discisigna* Hampson

209. *A. flava* Fabricius

210. *A. planalis* Swinhoe

211. *A. fulvida* Guenée

212. *A. banzigeri* Srivastava and Rose

213. *A. lyona* Swinhoe

214. *A. albitibia* Walker

215. *A. combinans* Walker

216. *A. mesogona* Walker
217. *A. involuta* Walker
218. *A. flava* Fabricius
219. *A. erosa* Hübner
220. *A. nigritarsis* Walker
221. *A. sabulifera* Guenée
222. *A. trilineata* Moore

Genus ***Rusicada*** Walker
Type species *Rusicada nigritarsis* Walker
223. *R. pindraberensis* Singh & Ranjan

Genus ***Arsacia*** Walker
Type species *Arsacia saturatalis* Walker
Synonyms: *Amblyzancla* Turner, *Notocyma* Snellen
224. *Arsacia rectalis* Walker

Genus ***Gnamptonyx*** Hampson
Type species *Alamis innexa* Walker
225. *Gnamptonyx innexa* Walker

Genus ***Sympis*** Guenée
Type species *Sympis rufibasis* Guenée
226. *Sympis rufibasis* Guenée

Genus ***Platyja*** Hubner
Type species *Phalaena umminia* Cramer
Synonyms: *Cotuza* Walker, *Ginaea* Walker, *Cremnodes* Felder, *Yerongponga* Lucas, *Mocrendes* Nye
227. *P. acerces* Prout
228. *P. ummina* Cramer
229. *P. exviola* Hampson
230. *P. torsilinea* Guenée
231. *P. ciacula* Swinhoe

Genus ***Amphigonia*** Guenée
Type species *Amphigonia hepatizans* Guenée
Synonyms: *Acygoniodes* Hampson
232. *Amphigonia hepatizans* Guenée

Genus ***Oxyodes*** Guenée
Type species *Noctua scrobiculata* Fabricius
233. *Oxyodes scrobiculata* Fabricius

Genus ***Hyperlopha*** Hampson
Type species *Ephyrodes cristifera* Walker
234. *H. cristifera* Walker
235. *H. crucifera* Walker

Genus ***Clytie*** Huebner
Type species *Noctua illunaris* Hübner
Synonyms: *Pseudophi***a** Guenee
236. *Clytie infrequens* Swinhoe

Genus ***Chrysopera*** Hampson
Type species *Achaea combinans* Walker
237. *Chrysopera combinans* Walker

Genus ***Pantydia*** Guenée
Type species *Pantydia sparsa* Guenée
Synonyms: *Rhiscipha* Walker, *Tantydia* Tillyard,
238. *Pantydia metaspila* Walker

Genus ***Rhabdophera*** Staudinger
Type species *Rhabdophera messrae* Staudinger
Synonyms: *Beriohansa* Nye
239. *Rhabdophera vetusta* Walker

Genus ***Drasteria*** Hubner
Type species *Drasteria graphica* Hübner
240. *Drasteria nephelostola* Hampson

Genus ***Anatatha*** Hampson
Type species *Catada nigrisigna* Hampson
241. *Anatatha nigrisigna* Hampson

Genus ***Sypna*** Boisduval & Guenée
Type species *Sypna omicronigera* Guenée
242. *S. dubitaria* Walker
243. *S. martina* Felder & Rogenhofer
244. *S. omicronigera* Guenée

Genus ***Ugia*** Walker
Type species *Ugia disjungens* Walker
245. *Ugia transversa* Moore

Genus ***Speiredonia*** Hübner
Type species *Phalaena feducia* Stoll
Synonyms: Sericia Guenée, Spiredonia Agassiz
246. *S. itynx* Fabricius

247. *S. mutabilis* Fabricius

248. *S. obscura* Cramer

249. *S. retorta* Clerck

250. *S. alix* Guené

The checklist of 250 species under 65 genera of Indian Erebinae will provide a first level list and act as a baseline for more detailed and comprehensive studies of the Erebinae.

ACKNOWLEDGEMENTS

The authors are grateful to the Head of the institution, Christ College (Autonomous) Irinjalakuda, Thrissur, Kerala, for providing facilities. The first author offers sincere gratitude to UGC, Government of India, for financial support in the form of UGC junior research fellowship (886/ (CSIR UGC NET JUNE 2018)

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(Received July 19, 2022; revised ms accepted November 18, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.797
ENTOMON 47(4): 433-436 (2022)
Short communication No. ent. 47410

Effects of magnetic field on the histology of silk gland of silkworm, *Bombyx mori* L. (Lepidoptera, Bombycidae)

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ABSTRACT: Magnetic field influences the physiology and development of living organisms, depending up on the strength of magnetic field. In silkworm, it enhances enzymes, proteins and nucleic acids of silk gland. On this line, histology of silk gland of silkworm *Bombyx mori* L. (Lepidoptera, Bombycidae) was studied after its magnetization, at 3500 G and 4000 G separately. Exposure of silkworm to magnetic field resulted in increase in diameter of its silk gland/lumen of silk gland/space occupied by secretory substance (silk protein-fibroin). The studies showed 46.15 and 21.19 per cent increase in diameter of silk gland of larvae exposed to 3500 G and 4000 G magnetic field respectively than that in control larvae. Larvae treated with 3500 G magnetic field and 4000 G magnetic field exhibited 51 per cent gain and 1.29 per cent loss in the size of secretory substance respectively than that of control group larvae. Cellular thickness is more in magnetized larvae than that of control larvae. This is favourable for sericulture.

KEYWORDS: Magnetization, physiological, cytoological changes

Sericulture is an important agro-based industry. More than 95 per cent of the total silk production of the world is from mulberry silkworm. In addition to industrial value, the mulberry silkworm *Bombyx mori* L. (Lepidoptera, Bombycidae) acts as laboratory tool in variety of research projects. This is because of its domestication, shorter life cycle with different metamorphic forms, considerable size, weight, easy to handle and good techniques of their culture. The environmental conditions and care taken during rearing of silkworm decides the quality and quantity of silk. Since many decades, efforts have been taken to enhance the silk producing capacity of silkworms by exposing them to various conditions of photoperiod, temperature, humidity, gamma rays, X-rays, amino acids and artificial diets (Chougale, 2003). Alterations in morphological (Gokcimen *et al.*, 2002), behavioural (Chougale, 2016), physiological (Conely, 1966; Pittman and Ormond, 1970; Ring, 1973), biochemical (Salem *et al.*, 2006; Elyamani 2020) and economical parameters have been reported in biological systems exposed to magnetic fields (Boe and Salunkhe, 1963). Magnetic field influences larval period and economic characters of cocoons (Chougale and More, 1992), enzymes (Chougale and More, 1993), nucleic acids (Chougale *et al.*, 1996), carbohydrates (Londhe *et al.*, 2021) of silk gland of silkworm and glycogen contents in tissue of pupae of silk moth (Prasad and Upadhyay, 2014).

Quality disease-free laying's (DFLs) of CSR × Kolar strain of silkworm were obtained from National Silkworm Seed Organization (NSSO),

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Mysore. The DFLs were incubated at 25°C and relative humidity 80-85 per cent was maintained. The larvae hatched from the DFL were supplied with V_1 variety of mulberry leaves and were reared separately under constant conditions of temperature and relative humidity. The rearing technique of Krishnaswami *et al.* (1973) was followed. On the first day, the 5th instar larvae were divided into three groups. One was reared as control and the two were used for magnetization and exposed to magnetic field as per procedure devised by Chougale and More (1992). Magnetization was done during the first three days of 5th instar by exposing the larvae for 20 minutes daily for magnetic field of 3500 G and 4000 G separately. They were kept in a perforated plastic container and it was placed between two poles of axial field electromagnet. The desired field strength was obtained by adjusting the distance between the poles. It was measured with digital Gauss meter. Five larvae from each experimental and control group were sacrificed. Using Traditional histology technique, posterior region of silk glands was fixed in 2 per cent calcium acetate formaldehyde (24 h), and after washing for 12 h they were dehydrated, cleared in xylene and embedded in paraffin wax (59-60°C). Then 0.6 µ transverse sections were taken using rotary microtome. Sections were dewaxed in xylene and stained with hematoxylin and eosin stains.

Transverse sections of posterior silk gland of each experimental group larvae showed alterations which were as follows:

1. The size/diameter of posterior silk gland of magnetized larvae was more than that of control group larvae. This was more pronounced in larval group magnetized at 3500 G than that of 4000 G.

2. The thickness of cellular layer of silk gland was more in experimental group larvae than that of control group larvae.

3. Size of secretory substance in lumen of silk gland was more in 3500 G magnetized larvae.

4. In larvae magnetized at 3500 G, nuclei of silk gland cell appeared more branched than in silk gland of control larvae (Fig. 1).

For conformation of above findings, efforts were made to study morphometry of different regions of T.S. of posterior silk gland. The studies showed 46.15 and 21.19 per cent increase in diameter of silk gland of larvae exposed to 3500 G and 4000 G magnetic field respectively than that in control larvae. Larvae treated with 3500 G magnetic field and 4000 G magnetic field exhibited 51 per cent gain and 1.29 per cent loss in the size of secretory substance respectively than that of control group larvae. Cellular thickness is more in magnetized larvae than that of control larvae.

Chougale (1992) have reported gradual increase in proteins and RNA of silk gland when larvae were exposed to 1000G to 3500G respectively. The magnetic field effect might be due to the change in the rate or pattern of translocation and accumulation of magnetically active microelements in cell and organ system (Mericle *et al.*, 1964). Low field strength is responsible for no effect or stimulatory ones, whereas, the higher field strengths result in inhibitory effects (Mulay and Mulay, 1964). Singh *et al.* (2003) and Elbaz and Ghonimi (2015), observed various histological changes in tissues of rats exposed to magnetic field. The electromagnetic energy and body of organisms has a valid and important relationship. Applications of magnetized water result in hyperplasia and DNA synthesis (Singh *et al.*, 2003). Instead of such hyperplasia, there may be enhancements of hypoploidy of silk gland in magnetized larvae than that of control larvae. Buntrock *et al.* (2012) have reported the small and more or less spherical nuclei in the silk glands of *Ephestia kuehniella*. However, nuclei of the late instar are irregular in shape and branched in nature. According to them, it is compensatory adaptation to improve molecular traffic between cytoplasm by enlarging surface to volume ratio of these nuclei. On this line in present studies, the magnetic field may have influenced the histological structures of silk gland and have resulted in more silk synthesis and secretion. Exposure of silkworm resulted in increase in diameter of its silk gland/ lumen of silk gland/space occupied by secretory

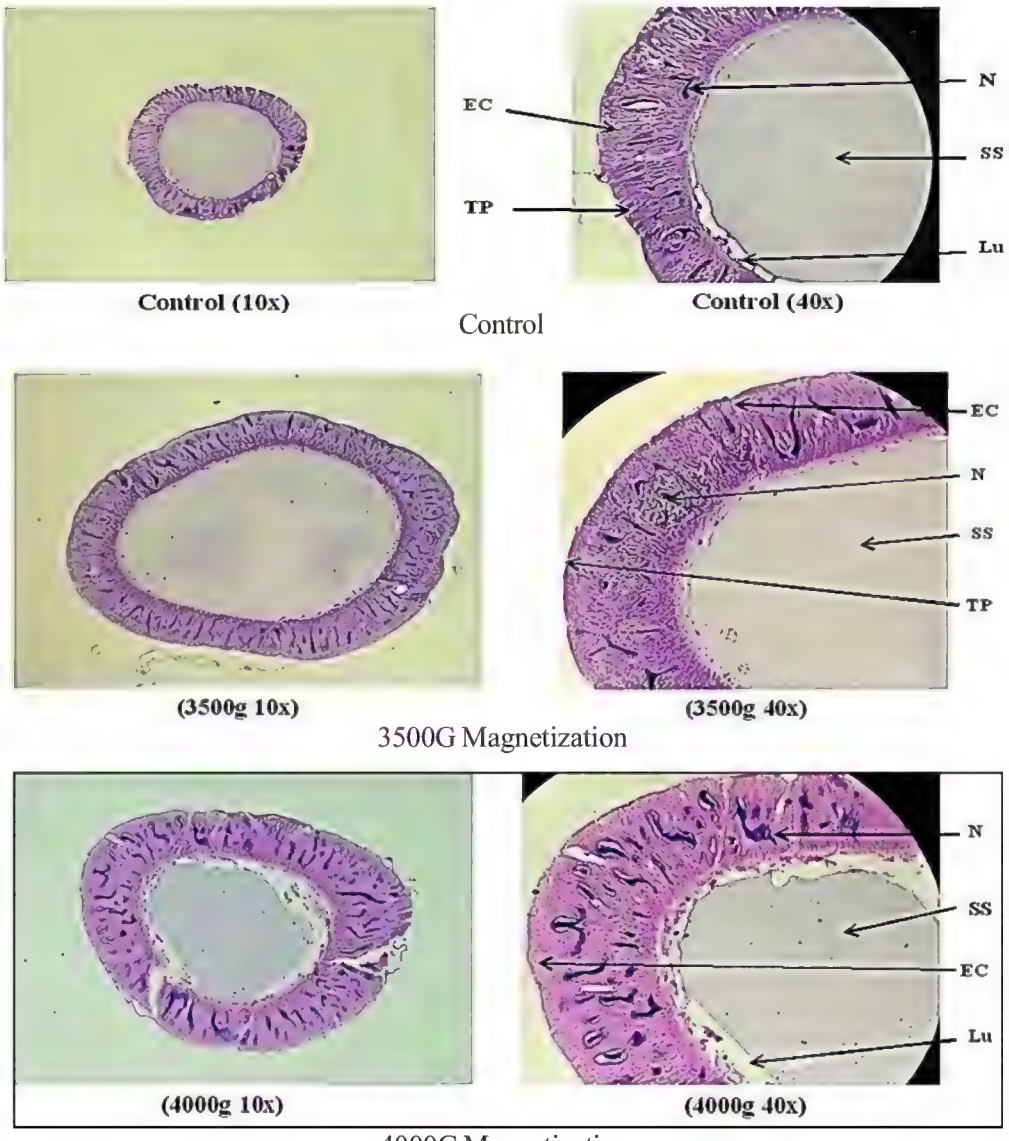

Fig. 1 T.S. of posterior region of silk gland of V instar silkworm
N: Nucleus, SS: Secretory Substances, Lu: Lumen, EC: Epithelial cells, TP: Tunica Propria

substance (silk protein- fibroin). This is favorable for sericulture.

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(Received August 08, 2022; revised ms accepted October 03, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.798
ENTOMON 47(4): 437-442 (2022)
Short communication No. ent. 47411

First record of cuckoo wasp *Trichrysis imperiosa* (Smith) (Hymenoptera, Chrysididae) from the nest of *Sceliphron coromandelicum* (Lepeletier) (Hymenoptera, Sphecidae) in India

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ABSTRACT: The present study could document, *Sceliphron coromandelicum* (Lepeletier) (Hymenoptera: Sphecidae) as the host of the cuckoo wasp, *Trichrysis imperiosa* (Smith) (Hymenoptera: Chrysididae) from Kerala, India. This is the first host record of *T. imperiosa*. Interesting observations and notes on their natural history are also reported. © 2022 Association for Advancement of Entomology

KEYWORDS: Host association, notes, natural history, Kerala, kleptoparasite

The Chrysididae popularly called "gold wasps" or "jewel wasps" are brightly coloured and shiny Hymenoptera, mostly brilliant metallic green, violet, gold and/or red (Rosa *et al.*, 2021a). They are also termed cuckoo wasps, since they use the nest of another species for laying eggs and rearing their own young. Evolutionarily they are specialized to defend themselves during oviposition; they curl into a defensive ball through conglobulation. Their strongly chitinized and sculptured body serve to defend the attack of their hosts (Houston, 2011). The natural history of chrysidid wasps remains poorly known, though they are widespread and are important natural enemies of several groups of Hymenoptera like Sphecidae, Eumeninae and Pompilidae (Kimsey and Bohart, 1991; Bank *et al.*, 2017; Sann *et al.*, 2018). Genus *Trichrysis* Lichtenstein, 1876 of subfamily Chrysidinae are parasitoids of sphecid or crabronid wasps (Rosa *et al.,* 2016) and also pompilids (Pärn *et al.,* 2015). The genus is distributed in Palaearctic, Afrotropical, Oriental and Australian Regions (Rosa *et al.,* 2016). A total of 121 species of Chrysididae under 20 genera and four subfamilies are known from India (Rosa *et al.*, 2021a; Rosa *et al.*, 2021b; Rosa and Halada, 2021; Aswathi and Bijoy, 2021).

The mud dauber wasp nest was collected from Pilassery, Kozhikode, Kerala (11.324°N; 75.9076°E) on 24-06-2021. The nest was initially kept for emergence; however, it was opened later for further studies. The fully developed individuals of both the sphecids and chrysidids were pinned and the rest were preserved in alcohol (70%). The mounted specimens were studied and photographed using Leica DFC 500 digital camera attached to Leica M205 A stereomicroscope (1X objective), and processed with LAS version 3.6, extended focus

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software. The voucher specimens are deposited in the National Zoological Collections of Zoological Survey of India, Kolkata.

The cuckoo wasp was identified as *T. imperiosa* (Fig. 1.a), with the help of taxonomic keys (Rosa *et al.,* 2021a). With five teeth on the apex of the 3rd metasomal tergite, it belongs to *T. lusca* species group. *T. imperiosa* is similar to *T. lusca* (Fabricius, 1804), however, differs in the colour of dorsal mesosoma, in the nature of frontal carina, and also the sculpture of second and third metasomal tergites. The body is metallic greenish-blue to blue and has golden reflections on face. The species is widely distributed in India and are documented from the states of Assam, Karnataka, Kerala, Maharashtra, Meghalaya, Sikkim, West Bengal and Arunachal Pradesh (Rosa *et al.*, 2021a). Elsewhere the species is known from China (Tsuneki, 1970); Australia, Myanmar, Sri Lanka (Bingham, 1903); Vietnam (Kimsey and Bohart, 1991); Indonesia, Nepal, Papua New Guinea and Thailand (Rosa *et al.*, 2016).

Morphological identification of *S. coromandelicum* (Fig. 1. b), was made using the key to species (Anagha *et al.*, 2021). It can be easily recognized by the pronotal collar with yellowish-brown band, black metasoma with fine setae and the yellow or yellowish-brown petiole. The species is distributed in Bangladesh, Cambodia, India, Laos, Malaysia, Myanmar, Sri Lanka, Thailand, Ukraine (Pulawski, 2021; Anagha *et al.*, 2021). In India, the species is documented from Andaman Islands, Assam, Bihar, Delhi, Goa, Himachal Pradesh, Karnataka, Kerala, Maharashtra, Meghalaya, Odisha, Pondicherry, Punjab, Sikkim, Tamil Nadu, Uttarakhand, Uttar Pradesh and West Bengal (Anagha *et al.*, 2021).

Natural history of *T. imperiosa* and *S. coromandelicum:* The female sphecids build their mud nests in a variety of sheltered and dry sites. They are also common in human habitations (Camillo, 2002). In the present study, the mud nests of *S. coromandelicum* were found on an unplastered, illuminated wall of a building, well protected from rain and sunlight, at a height of about 1.5 m from ground level. The nest had 10 subcylindrical cells, arranged in tiers (Fig. 1c), including an unopened cell. On breaking the single unopened cell, it was seen that the cells were provisioned with a host larva and 15 spiders, belonging to the families Clubionidae and Salticidae. Both juveniles and subadults (Fig. 1 d) could be seen. The host association of *T. imperiosa* could be ascertained since all the cells of the nest were not parasitized and three host sphecids too emerged from the same nest. In total, three *T. imperiosa* and two *S. coromandelicum* were found inside the sphecid nest (Table 1). It could be confirmed that similar to the host sphecid, only a single individual of chrysidid wasp developed from each cell. *T. imperiosa* individuals had constructed a mirror-like diaphragm across the center of the host cocoon which separated it from the host remains (Fig. 1. c).

Table 1. Details of the nest contents in each cell

No.	Cell content	Remarks
1	Vacant cell	Opened
2	Vacant cell	Opened
3	Vacant cell	Opened
4	*T. imperiosa*	Unopened
5	*T. imperiosa*	Unopened
6	*T. imperiosa*	Unopened
7	*T. imperiosa*	Unopened
8	*S. coromandelicum*	Unopened
9	*S. coromandelicum*	Unopened
10	15 spiders, *S. coromandelicum* larva	Unopened

All cuckoo wasps are parasitoids or kleptoparasites of Hymenoptera. They lay their eggs inside the host nest. In some species the hatched larva of the cuckoo wasp will consume the host larva when it is fully developed or in others it will start feeding the host egg or larvae as well as the provisioned food immediately after hatching (Szczepko *et al.,* 2003).

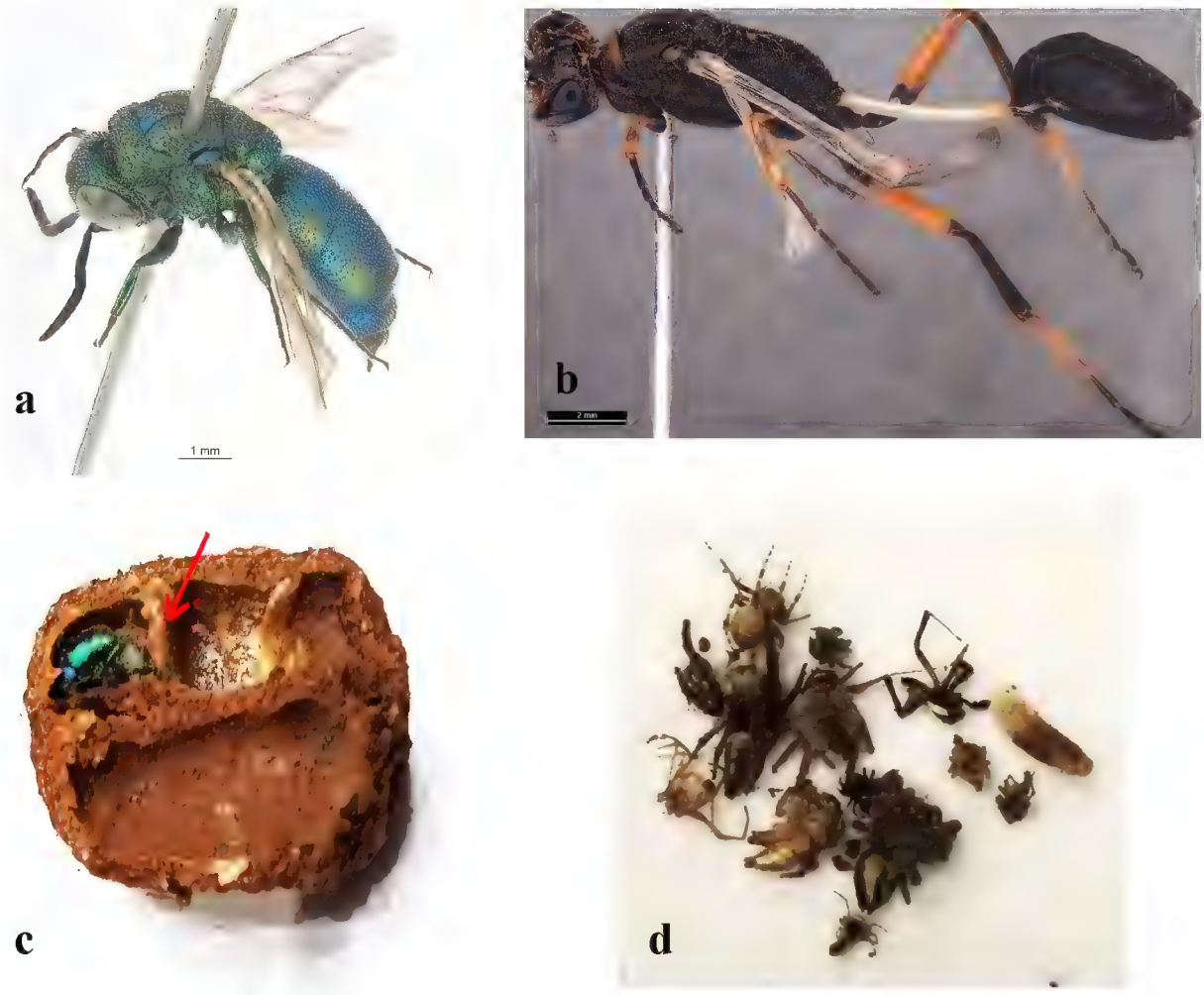

Fig. 1. a) *Trichrysis imperiosa*, b) *Sceliphron coromandelicum*, c) Cuckoo wasp inside *Sceliphron* nest with mirror like diaphram, d) Spider prey and larvae

Host-parasite associations of *Trichrysis* wasps have been documented by several authors (Dufour and Perris, 1840; García Mercet, 1911; Alfken, 1915; Enslin, 1921; Trautmann, 1927; Grandi, 1931, 1936; Danks, 1971; Groot, 1971; Lomholdt, 1975; Morgan, 1984; Kimsey and Bohart, 1991; Asís *et al.,* 1994; Kunz, 1994; Strumia, 1997; Rosa, 2006). Recently Pärn *et al.,* (2015) included some Pompilidae species as potential hosts for *Trichrysis*.

T. lusca is reported as a parasitoid of two species of *Sceliphron* - *S. fabricator* Smith (Mocsáry, 1889, 1912; Linsenmaier, 1959) and *S. inflexus* Sickmann (Tsuneki, 1955). A few unidentified species of Eumenidae were also documented as hosts of this species (Kimsey and Bohart, 1991). The cuckoo wasps with pollen-collecting species as hosts as in the case of bees may act as parasitoids rather than kleptoparasites (Pauli *et al.,* 2019). Accordingly, in the present study since the host species *S. coromandelicum* is not a pollen collector, it can be assumed that *T. imperiosa* is in the role of a kleptoparasite rather than a parasitoid. This is the first ever host record of *T. imperiosa* globally.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, Zoological Survey of India, Kolkata for providing facilities for accomplishing this work. The authors also thank Mr. Akhilesh Mangalassery, for his wholehearted support in the field collection. First and fourth authors are grateful to the Council of Scientific and Industrial Research for the Junior Research Fellowship.

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(Received July 04, 2022; revised ms accepted October 16, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.799
ENTOMON 47(4): 443-448 (2022)
Short communication No. ent. 47412

Additional record of the little known xylophagous endemic wood roach *Salganea rehni* Roth, 1979 (Blattodea, Blaberidae, Panesthiinae) from the Western Ghats, India with its DNA barcode

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ABSTRACT: The paper presents the record of the poorly known endemic species of wood roach from India after a gap of almost 40 years. In India, discernible work has been done on the DNA barcode of cockroaches including the genus *Salganea*. This work forms the first mitochondrial DNA barcode for the species *Salganea rehni* Roth, 1979. © 2022 Association for Advancement of Entomology

KEY WORDS: Rediscovery, India, Western Ghats

Cockroaches are phylogenetically closely related to termites. The diet of the termites is lignocellulosic while, cockroaches are omnivorous scavengers (Bell *et al.,* 2007). The blattid cockroaches have specialized gut microbiome to convert the lignocellulosic food material (Maekawa *et al.,* 2008, Schauer *et al.,* 2012). The genus *Salganea* was erected by Stål in 1877 under the subfamily Panesthiinae and the family Blaberidae (with the type species as *Panesthia morio* Burmeister, 1838). They live in the wood galleries tunneled in the rotten woods (Roth, 1979; Maekawa *et al.,* 2001) and are hence also considered as wood roaches. The lignocellulose digestion by these cockroaches helps in the turnover of organic matter in forest ecosystems (Roth, 1979). Some members from this genus live in biparental families having a male-female pair and the nymphs are fed by their parents (Maekawa *et al.,* 2005, 2006). Iteroparity and parental investments is believed to be the reason for lack of eusociality in *Salganea* (Maekawa *et al.,* 2008). The genus *Salganea* reproduces ovoviviparously, producing young ones by means of eggs that hatch within the body of the parent. Generally, wood roaches are heavily bodied insects, male and female have a similar pronotum (Maekawa *et al.,* 2008).

Accepting the underlying taxonomic instability with regard to number of species considered in the genus, Beccaloni (2007) suggested 47 species (and six subspecies) and Maekawa *et al.* (2001) and Nalepa *et al.* (2008) considered 50 species worldwide including 10 species from India (Gupta and Chandra, 2019). In the recent studies Beccaloni (2014), reported 50 species across the globe under the genus *Salganea*, which are further categorized into five morphological species groups.

Roth (1979) in his taxonomic revision of the Panesthiinae of the world reported 42 species and

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four subspecies under *Salganea*. In the same revisionary work, he erected five species-groups, based on the anterior margin of the pronotum and male genitalia. The five species-groups considered by Roth (1979) include the *papua* species-group, the *foveolata* species-group, the *raggei* species group, the *morio* species-group, and the *nigrita* species-group. Roth (1979) did not place 11 species in the above-mentioned species-groups including *S. rehni*. The morphological species-group created by Roth (1979), were not fully supported by the molecular phylogenetic studies conducted by Maekawa *et al.* (2001).

Very recently, an annotated checklist of cockroaches of India has been published (Gupta and Chandra, 2019) which has 10 species of *Salganea* including five-endemic species to Tamil Nadu region of Southern India (*Salganea cavagnaroi* Roth, 1979; *Salganea erythronota* Bolívar, 1897; *Salganea indica* Princis, 1953; *Salganea kodaikanalensis* Roth, 1979 and *S. rehni* Roth, 1979) (Fig. 2). Interestingly, all the endemic species are known from Tamil Nadu (Table 1) but the voucher specimens were not collected since their discovery after 1979. Among the 10 species known from India, *Salganea biglumis* (Saussure, 1895) is reported to have type locality in Sikkim, India with additional distribution records in Philippine Islands without any specific locality details (Roth, 1979). With this backdrop, the poorly known endemic species, *S. rehni* is reported here with the mitochondrial Cytochrome oxidase subunit I (mt COI) DNA barcode data from Agasthyamalai region of Upper Kodayar, south of the type locality, Annamalai region of Tamil Nadu, India (Fig. 2).

DNA barcoding and molecular studies in cockroaches / wood roaches in India are in a stage of infancy. India is known to have 181 species of cockroaches classified under 72 genera, 17 subfamilies and six families (Gupta and Chandra, 2019) of which 89 species are endemic to the country. Among the 181 species of cockroaches known from India, DNA barcode data (mt COI) is available for 33 species belonging to 23 genera in the GenBank.

In the present study, the *S. rehni* is identified by using morphological characters and for the first time an attempt was made to provide DNA barcode of

Table 1. *Salganea* species reported with their type localities (Roth, 1979)

No.	*Salganea* species	Type locality
1	*S. biglumis* (Saussure, 1895)	Sikkim, India
2	*S. cavagnaroi* Roth, 1979	Pykara, India
3	*S. erythronota* Bolívar, 1897	Madurai, India
4	*S. incerta* (Brunner, 1893)	Mooleyit, Burma
5	*S. indica* Princis, 1953	Anamalai Hills, India
6	*S. kodaikanalensis* Roth, 1979	Kodaikanal, Palni Hills, India
7	*S. morio* (Burmeister, 1838)	New Guinea
8	*S. passaloides* Walker, 1868	Ceylon, Sri Lanka
9	*S. raggei* Roth, 1979	Mt Angka, Thailand
10	*S. rehni* Roth, 1979	Attakati, Shola, India

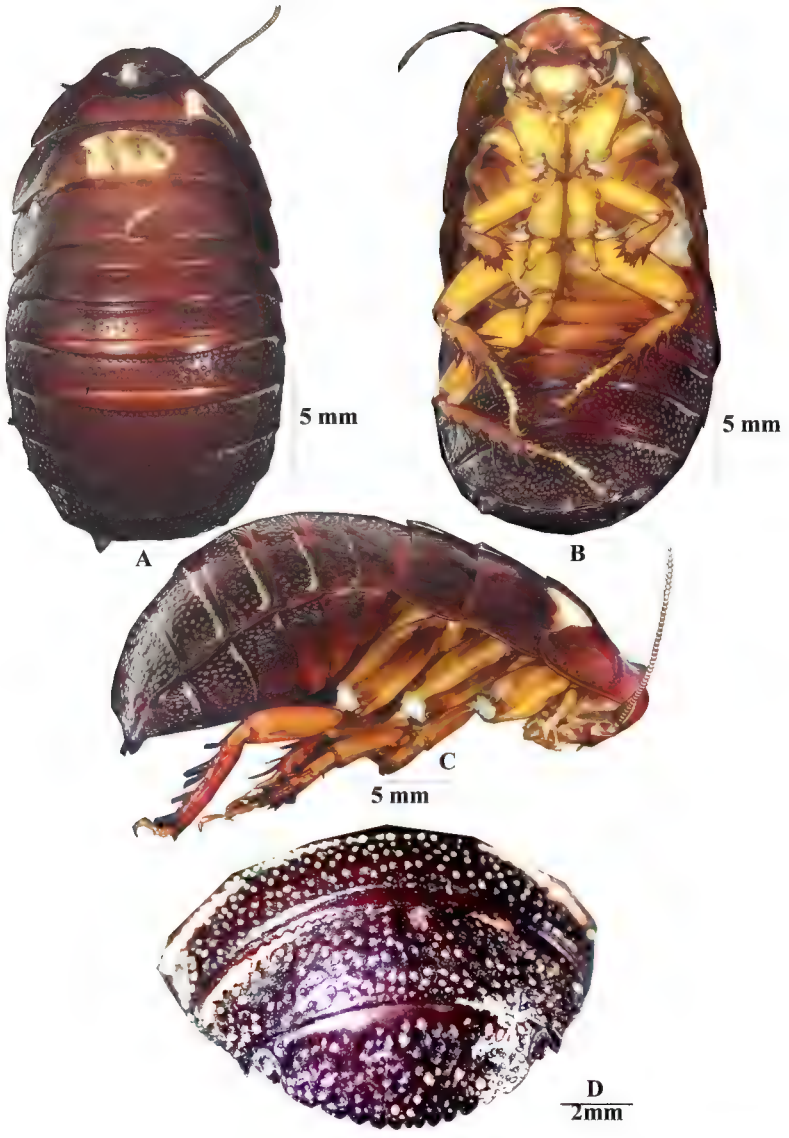

Fig. 1 *Salganea rehni*: Adult. A- Dorsal; B- Ventral; C- Lateral; D- Supra-anal plate

the voucher specimen collected in the recent field survey. Field sampling was done from decaying logs in the forest floors of Upper Kodayar (N 8.5377; E 77.3486; Altitude 1250 meters) (Fig. 2). The samples were preserved in ethyl alcohol for further studies. Among the two samples collected, the damaged sample was used for DNA studies and the specimen in good condition was used for both the DNA studies as well as taxonomic studies. Leica EZ4E stereomicroscope with photographic facility was used for examining the specimens and terminologies used follows Roth (1979). The identified specimen was deposited in the National Zoological collections of Zoological Survey of India, Western Regional Centre, Pune (ZSI, WRC). The map of the collection locality was prepared using open, free access QGIS software (Fig. 2). Genomic DNA was extracted with DNeasy Blood and tissue kit (Qiagen). PCR amplification was done using LCO1490 and HCO2198 primers (Folmer *et al.,* 1994) in 25 µL reaction volume including 12.5 µL of 2X master mix (Promega), 10 µM of each forward and reverse primer, 50 ng of template DNA and nuclease free water up to Q.S. Thermal cycle profile was as follows, one cycle of denaturation at 95^{0}C for 2 min; 5 cycles of 94^{0}C for 30 sec, 45^{0}C

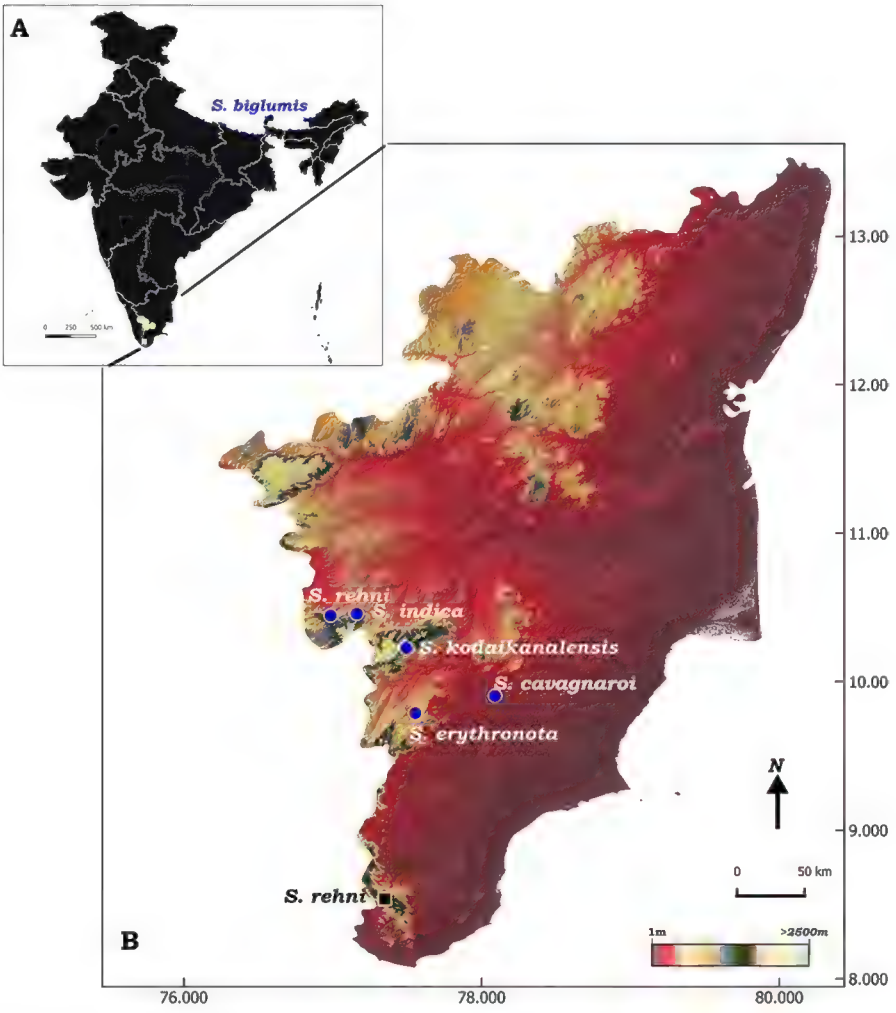

Fig. 2A - Type locality details for the species of *Salganea* described from India. B - Type locality of species described from Tamil Nadu (blue circle), and Upper Kodayar Tamil Nadu, the locality of *Salganea rehni* collected in present study (square)

for 1 min, 72°C for 1min; 30 cycles of 94°C for 30sec, 51°C for 1 min, 72°C for 1min followed by one cycle of final extension at 72°C for 5min (Hashemi-Aghdam *et al.,* 2017). Amplification band was confirmed by Gel electrophoresis stained with EtBr, followed by purification with Invitrogen's PureLink PCR Purification Kit. PCR product was sequenced by Sanger's method on ABI 377 (Applied Biosciences) sequencer. Morphologically the species could be identified as *S. rehni* Roth (1979) with an additional variation in the crenulation of supra-anal plate, shape of the teeth and a larger gap between the teeth (Fig. 1).

Taxonomic account

Order: Blattodea Brunner von Wattenwyl, 1882

Superfamily: Blaberoidea Saussure, 1864

Family: Blaberidae Saussure, 1864

Subfamily: Panesthiinae Kirby, 1904

Genus: *Salganea* Stål, 1877

1877. *Salganea* Stall, Ofvers. K. Sven. Vetenskapsakad. Foerh. 34, 33–58.

1903. *Mylacrina* Kirby, *Annals and Magazine of Natural History*, Series 7, 11, 404 - 415.

Type species. *Panesthia morio* Burmeister, 1838

Burmeister, 1838. Handbuch der Entomologie 2(2): 513.

Salganea rehni Roth, 1979

1979. *Salganea rehni* Roth, *Aust. J: Zool., Suppl. Ser.,* 1979, No. 69, 1-201.

Type locality. Inde meridionale, Attakatti, Shola am Iber Hill (= Tamil Nadu)

Material examined. ZSI-WRC Ent-12/82 24.viii.2019, 1 Female, Agasthyamalai Biosphere reserve, Upper Kodayar (N 8.537; N 77.348; altitude 1250 meters), Tamil Nadu, India, coll. K.P. Dinesh and Party.

Morphological description (Fig. 1A–D). Length-26mm. Body reddish brown, antennae brownish yellow, beaded and setose. Head sparsely punctate, vertex not foveolate, exposed. Pronotum convex, parabolic, anterior margin slightly indented, a pair of small reflexed tubercles behind the margin; anterior margin two-third depressed. Meso and metanotum sparsely punctulate. Tegmina and wings absent. Abdominal tergites hairless, T1-T7 punctate, T4-T7 densely punctate; Supra-anal plate (Fig. 1D) dense and coarsely punctate, rugulose, hind margin crenulate with 11-15 small subequal teeth which are broad at base and apically rounded. Abdominal sclerites punctate, denser on the lateral side, anterolateral corners of S7 with a small excavation lacking setae, lateral margin thickened under the cercus, hind margin rounded. Cercus with anterior margin curved, posterior apical angle broadly rounded. Anteroventral margin of front femur unarmed; distal spine absent; hind margin with a distal spine.

Original description of the species by Roth (1979) is based on female specimens and in the present study also only female specimens have been collected. As per the Paratype label the specimen was collected in 1921 from the Shambaganur region of Madurai parts of South India (with the register number BMNH (E) #876096; 130 km SW from type locality). Since the original description is from the Annamalai hill ranges and the current report of the species is from Agasthyamalai hills (around 190 km south of type locality), the species could be available in the hill ranges south of Palghat gap (Kerala and Tamil Nadu states).

The mt COI DNA barcode generated for *Salganea rehni* (Ent-12/82) is deposited in the GenBank (MW463933.1 and MW463934.1). Initial BLAST of the sequences MW463933.1 and MW463934.1 did not provide any 100 per cent match on NCBI. Since there is a dearth of DNA Barcode data for the cockroaches of Indian species, specifically for the genus *Salganea*, phylogenetic studies were not attempted. In the earlier studies Maekawa *et al.,* (2001) utilized mt COII gene for the study of 25 species of *Salganea* from the Southeast Asian region which is a partial range of distribution for the genus. Since the distribution range of the genus is wide spread, further studies are warranted from the South Asian region to understand the genetic pattern among the members.

Gupta and Chandra (2019) have included this species in their checklist but Prabakaran *et al.* (2019, 2020) did not include this endemic species in their document of Blattodea for the Tamil Nadu state, India. Most of the *Salganea* reports in India are based on the checklists without any voucher sample representation for any taxonomic or molecular studies. Present report of the little known xylophagous endemic wood roach, *Salganea rehni* from the Upper Kodayar is the first report of the species after its original description with a voucher specimen. Current mt DNA barcode forms the first mt DNA barcode for the species of *Salganea* as well as for the genus from India. The voucher specimen ZSI-WRC Ent-12/82 (Fig. 1) is expected to help the taxonomists in addressing the problems of Linnaean shortfall (Brown and Lomolino, 1998) and the mt DNA barcode generated is expected to support the understanding of Darwinian shortfall (Diniz–Filho *et al.,* 2013).

ACKNOWLEDGEMENTS

Authors are grateful to the Director, Zoological Survey of India, Kolkata and the Officer-in-Charge, Zoological Survey of India, Western Regional Centre, Pune for the facilities and support. Authors are thankful to Dr. Sameer Kumar Pati, Zoological

Survey of India, Western Regional Centre, Pune for his help in microscopic studies. Special thanks to Dr. R.M. Sharma Retd. Scientist, Zoological Survey of India, Western Regional Centre, Pune for critically going through the manuscript. Authors acknowledge the help of staff of ZSI, WGRC, Kozhikode and ZSI, SRC, Chennai for their support in field sampling. Authors thank Ms. Sangetha Devi and Sayali Aspate, Modern College of Arts, Commerce and Science, Shivajinagar, Pune, for their help in wet lab.

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(Received July 13, 2022; revised ms accepted October 01, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.800
ENTOMON 47(4): 449-452 (2022)
Short communication No. ent. 47413

New record of riffle bug *Rhagovelia* (*Neorhagovelia*) *nilgiriensis* Thirumalai, 1994 (Hemiptera, Heteroptera, Veliidae) from Kerala, India

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ABSTRACT: The riffle bug, *Rhagovelia* (*Neorhagovelia*) *nilgiriensis* Thirumalai, 1994 is reported for the first time from Kerala. They are very small, black bugs, commonly encountered in streams with moderate to swift flow. The current report of *R. nilgiriensis* from Kerala extends its geographic distribution which was earlier reported only from its type locality Nilgiris, Tamil Nadu, India. The present inventory is crucial, as it is the pioneer report of *R. nilgiriensis* from Kerala and the second record of the same from India.

KEYWORDS: First report, water bugs, Gerromorpha

The genus *Rhagovelia* Mayr, 1865 belong to the subfamily Rhagoveliinae, comprises a group of semi-aquatic bugs that are commonly known as riffle bugs, water crickets, small water striders, or broad-shouldered water bugs exclusively seen in lotic freshwater bodies with moderate to strong water current. The genus is the most specialized group among the veliids and also the most speciose in Gerromorpha, with around 400 described species (Polhemus, 1997: Zettel and Laciny, 2021). The members of the genus *Rhagovelia* are characterized by small size; elongated, cylindrical body; the last segment of middle tarsus is deeply cleft and bears a plumose swimming fan and leaf like claws arising from the cleft; mid femur and hind femur modified with several spine-like structures (Mayr, 1865). *Rhagovelia* have developed a considerable number of morphological modifications for facilitating rapid movements in swift running water (Andersen, 1976).Thirumalai (2002) recorded five species of *Rhagovelia* from India. Recently, several studies have been conducted on the taxonomy of aquatic and semi aquatic bugs in other states of India (Jehamalar *et al.*, 2018, 2019; Jehamalar and Chandra, 2020a, 2020b; Basu *et al.*, 2018; Bal and Hassan, 2021; Lyngdoh *et al.*, 2021). Unlike other states of India, systematic studies on aquatic and semi aquatic bugs from Kerala have been limited. An effort has been made in this investigation to document the aquatic bugs of a rivulet, Killiyar, in Thiruvananthapuram district of Kerala.

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As a part of the study, *Rhagovelia* (*Neorhagovelia*) *nilgiriensis* Thirumalai, 1994 was collected from Killiyar, in Thiruvananthapuram district of Kerala, by using a hand operated D-frame aquatic insect net with a mesh size of 500µm. The collected specimens were preserved in ethanol (70%) in the field and transported to the laboratory for detailed taxonomic studies. Male genital segments and its associated structures of the collected specimens were dissected and kept in potassium hydroxide (10%) for a period of 30 minutes for detailed examination. The photographs and measurements were taken using the Olympus TG- 6 digital camera and the Leica stereo zoom microscope (Leica M205A), using the software Leica application suite (Version 4.12). All measurements were taken in mm. Identification was done using the taxonomic literature (Thirumalai, 1994). The voucher specimens have been deposited in National Zoological Collection, Hemiptera Section, Zoological Survey of India, Kolkata, India.

Systematic account

Order Hemiptera Linnaeus, 1758

Suborder Heteroptera Latreille, 1810

Infraorder Gerromorpha Popov, 1971

Superfamily Gerroidea Reuter, 1910

Family Veliidae Amyot and Serville, 1843

Subfamily Rhagoveliinae China and Usinger, 1949

Genus ***Rhagovelia*** Mayr, 1865

Subgenus ***Neorhagovelia*** Matsuda, 1956

Rhagovelia (Neorhagovelia) nilgiriensis Thirumalai, 1994

1994. *Rhagovelia (Neorhagovelia) nilgiriensis* Thirumalai, *Rec. zool. Surv. India,* 94 (2-4): 390.

Material examined: Reg. No. 12415/H15, 2 apt.♂, 2 apt.♀, Killiyar, Thiruvananthapuram district, Kerala, 07.iii.2019, 8°32'44.45"N; 76°58'29.90"E, Coll. Jyothylakshmi K.

Diagnosis: Body length: 2.1- 2.8 mm; colour: black; except basal half of femora, yellow; body and legs clothed with minute setae; head and pronotum wider than long in males, wider in females; eyes black, antenna brownish black except, first segment basally yellow; coxa and trochanter of all legs yellow; fore femur shorter than tibia; mid femur is longer than fore femur and hind femur; hind femora with 1- 2 stout marginal spines in males; connexiva of female apically with short setal tuft reaching subapex of eight abdominal tergum; male paramere falciform sub basally with some scattered setae (Fig. 1 A- C).

Distribution: Known only from the type locality, India: Tamil Nadu.

Bionomics: *R. nilgiriensis* shows habitat and microhabitat specificity, restricted to rapidly flowing streams, cascades and riffles with sandy bottom. The species have morphological modifications such narrow; cylindrical body, modified legs; tuft of setae arranged in the form of a fan like structure in the last segment of the middle tarsus to facilitate swift movement in relatively fast-flowing streams. They are capable of avoiding capture by natural enemies by their swift movement. They were mostly collected from the cascade region of the stream. An interesting aspect of this species is the occurrence of macropterous and apterous adult morphs during different seasons. Most of the collected specimens were apterous and a single macropterous morph of female was obtained during the study. Furthermore, niche partitioning has been found, more males were commonly seen in swift flowing sections of the stream, while the females were mostly found in riffles near the shore. Like most other bugs, they are predacious in nature. More extensive observations are needed to reveal their life cycle and diet preferences.

Remarks: This is the first record of *R. (Neorhagovelia) nilgiriensis* Thirumalai, 1994 from Kerala. The species was so far exclusively documented from Tamil Nadu. This species can easily be separated from the other closely resembling species, *R.(Neorhagovelia) sumatrensis* Lundbald, 1936 by the presence of 1-2 marginal spines in the hind femora of males and by the shape of male paramere. The macropterous specimen was damaged and it has not been registered.

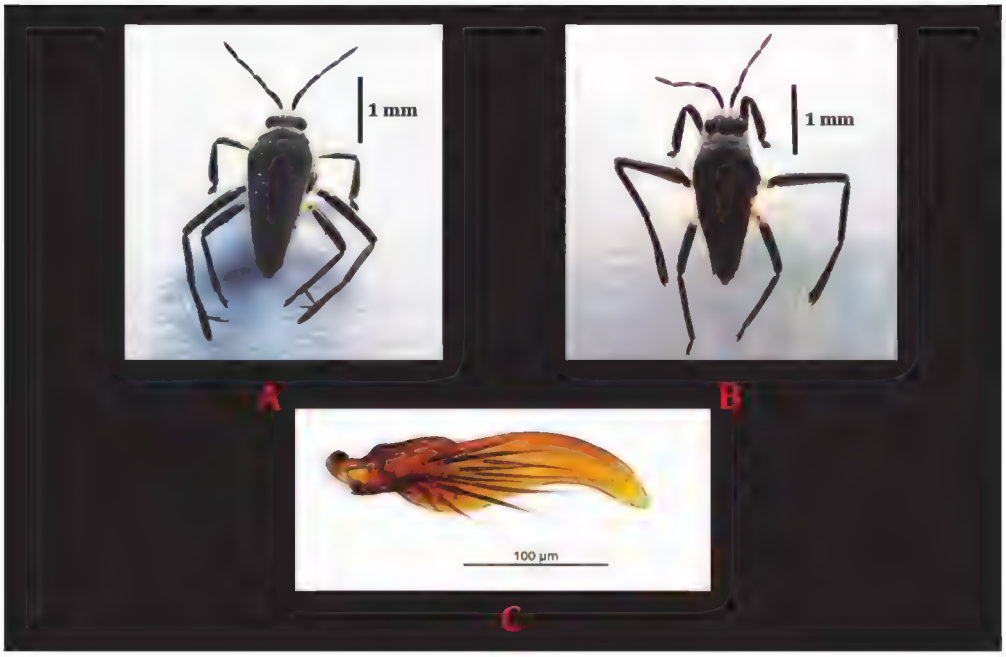

Fig. 1 *Rhagovelia (Neorhagovelia) nilgiriensis* Thirumalai, 1994 - A. Apterous male; B. Apterous female; C. Dorsolateral view of male paramere

The genus *Rhagovelia* Mayr, 1865 is the first most diverse genus among the semi aquatic bugs. Due to their poor dispersal abilities many *Rhagovelia* species are endemic to certain areas (Polhemus, 1995). The knowledge on the distribution of *R. (Neorhagovelia) nilgiriensis* is very poor. Thirumalai, 1994 described *R. (Neorhagovelia) nilgiriensis* from Tamil Nadu, India. The present inventory would be leading to fill the gap of information on its distribution data since it was not recorded from anywhere else in India after its first description by Thirumalai, 1994. Though the closely related species *R. sumatrensis* Lundbald, 1936 have several resemblances with *R. nilgiriensis,* the latter can easily be distinguished from the other by the narrow, falciform male paramere in contrast to subapically narrow male paramere of *R. (Neorhagovelia) sumatrensis* (Polhemus, 1990). *R. (Rhagovelia) tibialis* Lundbald, 1936 was the only known species of *Rhagovelia* in Kerala so far (Thirumalai, 1994; 2002). It can also be distinguished from the other species of *Rhagovelia* by the presence of parameres with blunt apex and short setae.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. A. Biju Kumar, Professor and Head, Department of Aquatic biology and Fisheries, University of Kerala, Kariavattom,Thiruvanathapuram, for the facilities and encouragement. E. Eyarin Jehamalar thanks Dr. Dhriti Banerjee, Director, Zoological Survey of India, Kolkata, West Bengal, for the facilities and also thank Dr. C. Raghunathan, Additional Director and Divisional-in-charge of Entomology Division-B., Dr. Swetapadma Dash, Scientist-E and Officer-in-Charge, Hemiptera Section, Zoological Survey of India, for their support.

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(Received June 07, 2022; revised ms accepted September 23, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.801
ENTOMON 47(4): 453-456 (2022)
Short communication No. ent. 47414

Antifeedant activity of aerial and root extracts of *Sphagneticola trilobata* (L) Pruski on *Spodoptera litura* (F.) (Lepidoptera, Noctuidae)

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ABSTRACT: Antifeedant activity of methanol and hexane extracts of aerial and root extract of *Sphagneticola trilobata* (L) pruski was tested against seven day old larvae of *Spodoptera litura* (Fab.) (Lepidoptera, Noctuidae) by no choice method of bioassay. A maximum antifeedant activity of 52.96 per cent was recorded at 0.1 per cent of methanol extract of aerial parts after 24 h of feeding. Root extracts exhibited low level of antifeedant activity against *S. litura*. At lower concentrations of 0.005, 0.01 and 0.03 per cent, there was no significant antifeedant activity. Antifeedant activity recorded after 48 h of feeding was similar to 24 h experiment but a slight reduction was noticed for 0.1 per cent of the methanol extract.

KEYWORDS: Leaf disc, choice bioassay, pre-starved larvae, crude extract, Asteraceae

Botanical pesticides are safe and effective alternatives to conventional pesticides, and they would help to reduce their use. Botanical pesticides have a number of properties that make them effective against agriculturally important pests, including pest toxicity, antifeedancy and insect growth regulatory activities. *Sphagneticola trilobata* (L.) Pruski is an herb included in the Asteraceae family that naturally grows in coastal regions, barren lands and forests, or as weed in crops, in many countries. This plant is also known as Singapore daisy, Wedelia, trailing or creeping daisy, water zinnia, and rabbits' paw in some countries (Meena *et al.*, 2011). Muscle cramps, rheumatism, stubborn burns, swellings, and arthritic swollen joints are all treated with *S. trilobata* in folk medicine (Arvigo and Balik, 1993). Junhirun *et al.* (2018) reported antifeedant activity of ethyl acetate extract of *S. trilobata* against *Spodoptera litura* (Fab), *S. exigua* (Hub) (Lepidoptera, Noctuidae) and *Plutella xylostella* (Lin) (Lepidoptera, Plutellidae).

The present study aimed at studying the antifeedant activity of methanol and hexane extracts of aerial and roots of *S. trilobata* against *S. litura*. Plant material collected from KAU campus were shade dried for two weeks. After the complete removal of moisture they were ground to fine powder and stored in zip lock cover at 4°C. Dried, powdered *S. trilobata* plant materials (100 g) were steeped in (300 ml) hexane and mixed properly by placing in a rotary shaker. After 24 h, the mixture was filtered through a Whatman No.42 filter paper and concentrated in vacuo in a rotary evaporator at a lower temperature. This process was performed

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three times to get crude hexane extract. The plant materials after extraction with hexane were subjected to re extraction with methanol (300 ml). Same procedure of extraction was followed as similar to hexane extract. After the removal solvents by rotary evaporator, crude methanol extract was obtained. Separate extraction was conducted for both aerial and root parts of *S. ttrilobata.* Methanol and hexane extracts of aerial parts were named as SP2 and SP1 and root extracts were named as SP3; SP4.

Castor leaf discs with a diameter of 4 cm were punched out from washed and dried castor leaves. Different concentrations of aerial and root extracts (0.005%, 0.01%, 0.03%, 0.05% and 0.1%) were made in carrier solvent. The punched-out castor leaf discs were thoroughly dipped in each concentration and air dried for one hour. Glass petri plates of 9 cm diameter were used for the experiment. Single treated leaf disc was placed at the centre of petri plate on which single prestarved one day old larvae of *S. litura* was released. Leaf disc treated with acetone was kept as control. Each treatment replicated 12 times. The leaf area consumed after 24 h of treatment was measured by using a mobile application (Easy7 leaf area free). Another set of experiment was kept and leaf area was measured after 48 h of feeding. Data was analysed in completely randomized design.

All the four extracts (SP1, SP2, SP3 and SP4) were sent to SAIF IIT, Bombay for GCMS and LCMS analysis. GCMS analysis was done for hexane extracts and LCMS was done for methanol extract. Major compounds present in the extracts were recorded. Antifeedant activity of various extracts of *S. trilobata* was tested against *S. litura* by no choice method. Concentrations ranging from 0.005 to 0.1 per cent were evaluated for 24 and 48 h of exposure. Among different extracts methanol extract of aerial parts exhibited maximum antifeedancy of 52.96 per cent at 0.1 per cent of the extract after 24 h of feeding. For all other extracts an antifeedant activity, less than 40 per cent was recorded even at higher dose. Activity was high in methanol extracts compared to hexane extracts. Lowest antifeedant activity was exhibited by hexane extract of roots. On comparing the activity of roots and aerial parts, aerial parts were superior in nature. At the lowest concentration of 0.005 per cent hexane extract of aerial parts exhibited higher antifeedant activity than other three extract. The decreasing order of antifeedancy of

Table 1. Antifeedant activity (%) of various extracts (SP1 to SP4) of *Sphagneticola trilobata* against 7 day old larvae of *Spodoptera litura* at 24 and 48 h

Conc (%)	SP 1		SP2		SP3		SP4	
	24h	48h	24h	48h	24h	48h	24h	48h
0.005	9.462^{b}	4.974^{c}	5.169^{d}	6.76^{d}	3.21^{d}	4.53^{d}	6.693^{c}	7.96^{d}
0.01	10.00^{b}	10.08^{bc}	16.07^{c}	15.12^{c}	3.16^{d}	9.44^{c}	9.287^{c}	9.70^{d}
0.03	17.25^{b}	11.37^{b}	21.64^{c}	21.05^{bc}	12.38^{c}	16.92^{b}	19.88^{b}	17.47^{c}
0.05	19.13^{a}	24.17^{a}	31.23^{b}	27.28^{b}	21.56^{b}	22.31^{b}	24.96^{b}	26.18^{b}
0.1	39.67^{a}	35.83^{a}	52.96^{a}	51,09^{a}	33.49^{a}	30.67^{a}	36.10^{a}	33.76^{a}

Figures in the column followed by same letter is not significantly different at $p < 0.05$ by Tukey's test

four extract was SP2>SP1>SP4>SP3. Similar results were obtained after 48 h of feeding. Maximum antifeedant activity was recorded for SP2 (51.9 %) at highest dose of 0.1 per cent. At lower concentrations no significant difference in activity was recorded for any of the extract. For all the extracts, antifeedant activity reduced after 48 h of exposure at highest concentration of 0.1 per cent. Similar to 24 h treatments, root extracts exhibited lower antifeedant activity compared to aerial parts.

LC/MS analysis of methanol extract of *S. trilobata* showed the presence of 12 phytochemical compounds; Xylitol, de-hydro epi androsterone, andrographolide, genistein, taxifolin, emodin, galangin, methyl caffeate, (-) - Caryophyllene oxide and artemisinin. Compounds like artemisinin, andrographolide, taxifolin and galangin *etc.* identified from methanol extract of aerial parts have previous record of antifeedant and growth inhibitory activities against many phytophagous insects. While in GCMS analysis of hexane extracts, only fewer compounds were reported.compared to aerial extracts, active molecules were less in root extract.

The present result closely matches with the findings of Junhirun *et al.* (2018) who reported that methanol extract was superior with a median antifeedant index of 0.33 mg ml^{-1} and 9.47 mg ml^{-1} against *P. xylostella* and *S. litura* respectively. The results are in close proximity with Pathrose *et al.* (2011) who evaluated antifeedant activity of andrographolide by no choice method against *S. litura* and recorded a maximum antifeedance (64.20% at 0.1 % concentration after 24 h of feeding). Similarly, antifeedant activity of artemisinin was evaluated by Maggi *et al.* (2005) by no choice method against *S. eridania* and recorded a maximum antifeedance of 87 per cent at 1.5 mg per ml of the test compound.

Wang *et al.* (2009) reported methanol extract of *Wedelia chinensis* for its antifeedant activity against third instar larvae of *S. litura* by no choice method of bioassay and recorded a gradual increase in antifeedant activity from lower concentrations to higher concentrations and a highest antifeedant activity of 90 per cent was recorded at 5 per cent concentration of extract of *W. chinensis*, corroborating the present observations. Similarly 80 per cent antifeedant activity was obtained at 1 per cent methanol extract of *W. chinensis* against larvae of *Cnaphalocrosis medinalis* (Qinglong *et al.,* 2012) and supports the present findings. Reduced antifeedant activity of root extract of *S. rilobata* was agreeable with the findings of Cayiun *et al.* (2006), where they reported lower antifeedant activity for roots (AFC50 = 6618.8 µg ml^{-1}) of *W. chinensis* against *Ostrinia furnacalis* compared to aerial parts including flowers (AFC50 = 3408.31µg ml^{-1}). Reduced antifeedant activity *S. trilobata* root extracts might be due to presence of less bioactive molecules in roots compared to aerial parts.

ACKNOWLEDGEMENT

This forms part of M. Sc. (Ag) thesis submitted to the KAU by the senior author. The senior author gratefully acknowledges award of KAU fellowship during the study period.

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(Received July 13, 2022; revised ms accepted September 21, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.802
ENTOMON 47(4): 457-462 (2022)
Short communication No. ent. 47415

Diversity and community structure of Ephemeroptera, Plecoptera and Trichoptera in Kolli hills of the Eastern Ghats, India

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ABSTRACT: The study describes the diversity and community structure of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa present in the Puliyancholai stream of the Kolli hills, Eastern Ghats. During the six months of study 397 specimens from 11 genera under seven families were collected. Ephemeroptera was the most dominant species followed by Trichoptera and Plecoptera. Various alpha biodiversity indices showed that the Simpson's index was maximum in October (0.878) and minimum in December (0.832). The Shannon-Weiner index was maximum in December (2.277) and minimum in January (2.151). Evenness index was most noteworthy in October (0.872) and it was least in December (0.725). Temperature, pH, calcium and magnesium are major stressors in governing the EPT community of Kolli hills, according to Canonical Correspondence Analysis (CCA). © 2022 Association for Advancement of Entomology

KEY WORDS: EPT taxa, biodiversity indices, Canonical Correspondence Analysis

Biodiversity refers to the variety of species, ecological variation, and genetic variation in a given ecosystem. Diversification is an important part of maintaining a healthy environment. Every species in an ecosystem plays an important function and is dependent on one another for their survival. Streams are physically diverse ecosystems that include a vast range of water habitats, ambient conditions, and biotic creatures. Anthropogenic activities have put the freshwater ecosystems under a variety of stresses. As a result of this, both the aquatic life and the human population are threatened. Freshwater benthic macro invertebrates such as Ephemeroptera, Plecoptera and Trichoptera (EPT) serve as the model organisms for meeting the ecological demands of the freshwater ecosystem (Beauchard *et al.,* 2003). Water flow, temperature, seasonality, altitude, pH, and dissolved oxygen are some of the ecological factors that influence the aquatic insect diversity and its community structure (Hodkinson and Jackson, 2005). Deterioration of freshwater is a case of concern mostly for the developing countries and it's a subject of debate, the study of aquatic organisms and their diversity can give us critical information regarding the water and ecosystem quality for the present and it will

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help us to do several actions to safeguard the freshwater ecosystems in the future if it is needed. In this context, an effort was made to start documenting the EPT fauna of the Puliyancholai stream, Kolli hills, Namakkal District, Tamil Nadu.

Sampling and collection of EPT taxa: Puliyancholai is located at the foot slopes of Kolli hills, Tamil Nadu. This region has rich green vegetation, trees, and Tamarind forests. It is located 241 km from Madurai and 76 km from Trichy District and latitude-longitude is 11°362' 01" N; 78°33' 03" E. The present study was carried out from October 2019 to March 2020 in Puliyancholai stream.

The random sampling was done from October 2019 to March 2020; it is because the falls is usually dry during other seasons. The nymphs of Ephemeroptera, Plecoptera, and Trichoptera were collected from the Puliyancholai stream of the southern Eastern Ghats. EPT complex was sampled by using a Kick-net (Burton and Sivaramakrishnan, 1993) with a mesh size of about 1mm and stored in ethyl alcohol (99.9%). EPT samples were examined under a stereomicroscope (Magnus Pro) and identified using standard taxonomic literature (Barathy *et al.,* 2021a). Water temperature, air temperature, pH, water flow, dissolved oxygen and turbidity were measured and analyzed using the APHA guidelines (APHA, 2005). PAST 4.0 version was used to analyze the data and calculate the Shannon, Simpson, and Evenness indices (Hammer *et al.,* 2001). Canonical Correspondence Analysis (CCA) was also done using the PAST software to find the relation between EPT insects and environmental attributes (Ter Braak and Smilauer, 2002).

Diversity and distribution of Puliyancholai stream: During six months, 397 EPT taxa were collected under 11 genera and 8 families. Baetidae, Caenidae, Hepatageniidae, Leptophlebiidae, Tricorythidae, Perlidae, Hydropsychidae and Stenopsychidae were families present in the Puliyancholai stream (Table 1). According to Selvakumar *et al.* (2012) the presence of *Baetis* sp., *Afronurus kumbakkaraiensis* Venkataraman and Sivaramakrishnan, 1989, *Epeorus petersi* Sivaruban, Venkataraman and Sivaramakrishnan, 2013 (Sivaruban *et al.,* 2013), *Thalerosphyrus flowersi* Venkataraman and Sivaramakrishnan, 1987 and *Choroterpes alagarensis* Dinakaran, Balachandran and Anbalagan, 2009 are useful as bioindicators of forest conditions. Heptageniidae, Baetidae, and Tricorythidae were the more abundant and widespread in the present sites. Suhaila and Che Salmah (2010) stated that survival of *Baetis* sp. and *Thalerosphyrus* sp., was greater during the rainy season, confirming that these species were well suited to broad substrates and swift currents. Shannon Weiner's index ranges from 2.151 to 2.277 and was found to be maximum in December and October and least in January. According to Javaid and Ashok (2013), Shannon-Wiener diversity values ranging from 1 to 2 imply highly contaminated water. In this study, the majority of the diversity index values recorded from the study sites ranged above 2. As a result, it was found that the Puliyancholi stream was moderately polluted by anthropogenic activities. The Simpson index ranges from 0.832 to 0.878, with October being the most extreme and December being the least. In present study the Evenness index ranged from 0.725 to 0.872 indicating the uniform distribution of insects in the community (Table 2). In the present investigation, high air temperature (30^0C) and water temperature (26^0C) were recorded in February and March. Corbet (2004) reported that warm water has been shown to have low dissolved oxygen content. Barathy *et al.* (2021b) reported that high water and air temperatures lead to a decline of low tolerant taxa. Normal dissolved oxygen (DO) level in the freshwater streams was found to be 4.6 - 8.6 mg L^{-1} (Srinivasan *et al.,* 2019) and low DO reduces the EPT richness, whereas, high DO nourish the EPT taxa. Similar results were found in the present study sites, the DO was maximum (9.3) in December and minimum in January (7.3). Breitburg (2002) stated that oxygen concentrations in aquatic ecosystems changed periodically, with winter being greater than summer. The pH range of 6.5 to 8.0 offers acceptable protection for freshwater fish and bottom-dwelling macro invertebrates. The pH levels at the present study sites are in the acceptable range of 7.1 to 8.1 (Table 2). Alkalinity values of 20-200 mg L^{-1} are

Table 1. EPT taxa recorded in the Puliyancholai falls, India during 2019-2020

Order/ Family	Genus/species	Oct	Nov	Dec	Jan	Feb	Mar
Ephemeroptera/ Baetidae	*Acentrella vera* Müller-Liebenau, 1982	9	8	7	8	7	6
	Centroptella ghatensis Kluge, 2021	5	4	3	6	5	6
	Nigrobaetis paramakalyani Kubendran and Balasubramanian, 2015	6	4	5	5	6	6
	Labiobaetis sp.	5	5	5	4	6	5
Ephemeroptera/ Caenidae	*Caenis* sp.	3	2	2	3	2	3
Ephemeroptera/ Heptageniidae	*Afronurus kumbakkaraiensis* Venkataraman and Sivaramakrishnan, 1989	17	18	18	19	18	20
Ephemeroptera/ Leptophlebiidae	*Choroterpes alagarensis* Dinakaran, Balachandran and Anbalagan, 2009	8	6	9	10	8	9
Ephemeroptera/ Tricorythidae	*Sparsorythus sivaramakrishnani* Sivaruban, Srinivasan, Barathy, Bernarth-rosi and Isack, 2021	5	4	5	4	5	3
Plecoptera/ Perlidae	*Neoperla biseriata* Zwick, Anbalagan and Dinakaran, 2007	4	5	3	2	4	6
Trichoptera/ Hydropsychidae	*Hydropsyche* sp	0	2	2	1	3	2
Trichoptera/ Stenopsychidae	*Stenopsyche kodaikanalensis* Swegman, 1980	2	4	4	5	6	5

common in freshwater environments. In the present study sites, total alkalinity was highest (69) in February and lowest in October (41) which support the growth of EPT taxa. In the stream, the total hardness was elevated in January (49) and it was low in October (23). According to Bispo *et al.* (2006) a rapid increase in water flow promotes stream bed translocation, which results in the removal of insects and a decrease in their local abundance. In the present study, the highest water flow was recorded in October (0.65) and the lowest was recorded in March (0.92). According to Resende *et al.* (2021), high water flow intensity and frequency can cause rapid declines in aquatic biodiversity species richness and abundance. The results of the study areas show that the concentration of calcium was highest in November (59) and lowest in February (42). Magnesium is required by most forms of life, including aquatic organisms (Maret, 2016) because of their high enzymatic functions, these metals play an important metabolic role in the bodies of organisms, particularly in regulating aquatic insect homeostasis. In the present study, magnesium was maximum in February (7.6) and minimum in October (2.5). Turbidity was maximum in November (0.9) and minimum in October (0.5). According to Mahajan and Billore (2014), water transparency is inversely proportional to turbidity, which is caused by suspended particles and organic matter, planktons and other microscopic organisms.

CCA results: As per CCA analysis (Fig.1) various physicochemical parameters have influenced the diversity and distribution of the EPT community. The CCA biplot reveals that increasing water temperature characterized the distribution of the genera *Caenis sp.* and *Centroptella ghatensis* Kluge, 2021. High DO and pH support the growth of *A. kumbakkaraensis*. According to Sivaruban *et al.* (2020a) stoneflies and heptageniids prefer cool environments and require oxygen rich

Table 2. Diversity indices of EPT taxa and physico-chemical parameters of water sample in Puliyancholai stream, Eastern Ghats, India

Parameters	Oct	Nov	Dec	Jan	Feb	Mar
DO (mg L^{-1})	8.4	8.1	9.3	7.3	7.5	7.4
Calcium (mg L^{-1})	57	59	53	51	42	50
Magnesium (mg L^{-1})	2.5	3.2	4.0	7.5	7.6	4.7
pH	7.1	8.1	8.1	7.3	7.1	7.4
Total alkalinity (mg L^{-1})	41	53	52	69	67	66
TDS (mg L^{-1})	57	69	69	83	84	76
Total Hardness (mg L^{-1})	23	24	26	49	48	45
Turbidity (NTU)	0.5	0.9	0.8	0.7	0.8	0.7
Air temperature (^{0}C)	29	29	28	30	30	30
Water temperature (^{0}C)	26	25	25	27	28	28
Water flow (m S^{-1})	0.65	0.74	0.78	0.82	0.85	0.92
Simpson index (H)	0.878	0.849	0.832	0.876	0.874	0.876
Shannon index (1-D)	2.261	2.256	2.277	2.151	2.253	2.246
Evenness (E)	0.872	0.785	0.725	0.872	0.864	0.859

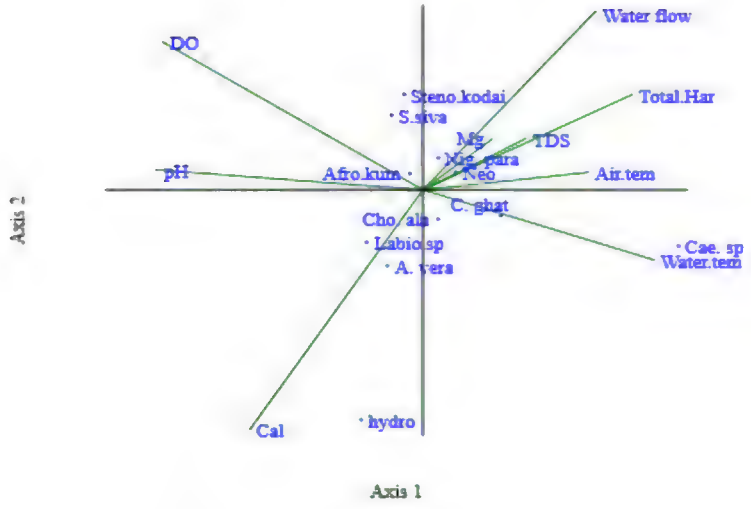

Fig. 1 Canonical correspondence analysis of Puliyancholai stream, India

(A.vera- *Acentrella vera*, Labio sp- *Labiobaetis* sp, Afro.kum- *Afronurus kumbakkarensis*, C.ghat- *Centroptella ghatensis*, Cae.sp- *Caenis* sp, Cho.ala- *Choroterpes alagarensis*, Nig. para- *Nigrobaetis paramakalyani*, Neo- *Neoperla* sp, Steno.kodai- *Stenopsyche kodaikanalensis*, S.siva- *Sparsorythus sivaramakrishnani*, hydro- *Hydropsyche* sp, DO- Dissolved Oxygen, cal-calcium, Mg- magnesium, Total.Har- total hardness, Water.tem- water temperature, Air.tem- air temperature)

environments to survive. High DO and water flow promote the growth of *Stenopsyche kodaikanalensis* Swegman and Coffman 1980 and *Sparsorythus sivaramakrishnani* Sivaruban, Srinivasan, Barathy, Bernarth-rosi and Isack, 2021 which are extremely sensitive to changes in the water temperature. The CCA results in the EPT community of Kiliyur falls, of Eastern Ghats, India showed that temperature, dissolved oxygen and rainfall turns into a major stressor (Sivaruban *et al.*, 2020b). *C. alagarensis*, *Labiobaetis* sp., *Acentrella vera* Müller-Liebenau, 1982 and *Hydropsyche* sp., prefers high level of calcium and are sensitive to high levels of air temperature, total hardness and TDS. High levels of water flow, TDS, total hardness, air temperature, and magnesium supports the growth of *Nigrobaetis paramakalyani* Kubendran and Balasubramanian,

2015 (Kubendran *et al.,* 2015) and *Neoperla biseriata* Zwick, Anbalagan and Dinakaran, 2007 while calcium was negatively related. The correlation coefficient between species and site scores is equal to the Eigen values associated with each axis. Thus, an Eigen value close to represents a high degree of correspondence between species and sites, whereas an Eigen value close to zero represents very little correspondence reported by Barman and Gupta (2015). The sum of all Canonical eigen values found in this study was axis 1 is 52.55 per cent and axis 2 is 22.82 per cent, indicating a high degree of correspondence of species with seasons.

This study revealed that *A. kumbakkaraiensis*, *S. sivaramakrishnani*, *C. alagarensis* and *A. vera* are the most dominant taxa in the Puliyancholai stream of the Eastern Ghats and environmental factors such as DO, pH, water flow, turbidity, air temperature, and water temperature are the major stressors governing EPT distribution, and the EPT's diversity decreased with the anthropogenic effect. This is comparable to that observed in previous studies that assessed the impact of anthropogenic pressures on aquatic insect biodiversity (Srinivasan *et al.,* 2019, Ligeiro *et al.,* 2013; Bijita and Susmita, 2015; Ramezani *et al.,* 2016). In the present study fewer genera were found due to anthropogenic activity.

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(Received July 31, 2022; revised ms accepted October 23, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.803
ENTOMON 47(4): 463-468 (2022)
Short communication No. ent. 47416

Adverse effects of cyfluthrin on *Cyphoderus javanus* Borner (Collembola) in soil

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ABSTRACT: Soil collembolans are key model organisms for ecotoxicological studies and play an inevitable role in litter degradation, nutrient cycling, energy flow and various ecosystem functioning. The detritivore collembolan, *Cyphoderus javanus*, was used to determine the toxicity of insecticide formulation cyfluthrin under laboratory conditions. The impacts of insecticide cyfluthrin on life history parameters of *C. javanus* revealed that mortality rates increased with increasing concentration. The fecundity rates, the number of eggs laid, the number of juveniles' emergence and longevity were found to be decreased drastically with insecticidal exposure. The high mortality of soil collembolans deducts the decomposition rate of organic matter and leaf litter, thereby reducing the fertility of soil.

KEYWORDS: Springtails, bioaccumulation, life history parameters

Soil is a complex living entity that breaths, assimilates organic and inorganic elements, breakdowns and mineralizes organic matters of biological origin, and stores reserves as organic matter (Sharma and Parwez, 2017). In most soils, 90 per cent of the soil micro arthropod population is composed of Collembola and Acarina (Wallwork,1976) and are of immense importance in major soil processes such as humification, recycling, mineralization of organic matter, mechanical decomposition of organic residues, stabilization of soil aggregates and pedogenesis (Emmerling *et al*., 2002). Collembola, commonly known as springtails, are small wingless, soft-bodied hexapods, usually found on or near soil surface, beneath rocks and the bark of trees (Paul *et al.,* 2011). These highly abundant groups of soil-dwelling micro arthropods can positively influence soil structure and functioning by modifying soil's biological, physical and chemical properties (Haque, 2018). Soil invertebrate communities, especially springtails, are crucial for monitoring the impacts of agricultural practices on environmental quality and soil functioning and are also regarded as valuable bio indicators to evaluate soil quality in human-altered systems (Velasquez *et al.,* 2007, Rousseau *et al*., 2013, Demetrio *et al.,* 2020). The most active detritivore collembolan, *Cyphoderus javanus* Borner, is considered an ideal potential biological marker of soil quality and ecosystem stability. The indiscriminate use of synthetic and organic pesticides, inorganic fertilizers and other agrochemicals resulted in the deterioration of crop yield, soil texture, disturbance of non-target

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organisms and ecological equilibrium of agricultural lands, mainly in tropical regions (Saha and Joy, 2014). A study by Thompson and Gore (1972) reported that springtails are highly susceptible to pesticides. Studies on the toxic impacts of pesticides and bioaccumulation of heavy metals in acarians, isopods and collembolans were reported earlier. Notable contributions among them are Mohammed *et al.*, 1992; Park and Lees, 2005; Greenslade *et al.,* 2010; Vinod and Sanalkumar, 2017; Niemeyar *et al.,* 2018; Zhang and Filser, 2020. In India, limited data exist on toxicity works related to feeding, hatching and development of soil arthropods. Therefore, it is essential to carry out more research on eco-toxicological effects on various aspects of soil fauna, particularly for collembolans. Cyfluthrin a pyrethroid insecticide was chosen for the study. The adverse effects of cyfluthrin on mortality rate, and life history parameters (fecundity, juveniles' emergence and longevity) of *C. javanus* were investagated.

Experimental organisms, *C. javanus*, for the present investigation, were collected from three different sites in the Thiruvananthapuram district - Neyyar, Vithura and Agasthyavanam biological park. Sufficient soil samples of 5× 5 cm^2 from a depth of 0-10, 10-20 and 20-30 cm were randomly collected using a soil auger and taken to the laboratory in a labelled polythene cover. Extracted soil micro arthropods were carried out by Berlese Tullgren Funnel, and micro arthropods extracted overnight into a picric acid medium (Haarlov, 1947). Polythene rearing jars of 7×3 cm were used to maintain stock culture for experiments. Eggs of *C. javanus* from the culture were separated, and a group of five each were kept in separate replicate culture chambers.

Bioassay studies: Adult collembolans were collected in a separate culture chamber and fed with decayed jackfruit leaves for seven days for acclimatization. The culture chamber was moistened with a wet cotton plug and kept in one corner of the culture chamber. Cyfluthrin of 5, 12, 14, 18, 20 and 22 ppm concentrations were prepared by dissolving an appropriate amount of the chemical in one litre of distilled water (APHA, 2012). Adult collembolans were exposed to each concentration of cyfluthrin in different culture chambers. Decaying leaves washed in water and soaked in respective agrochemicals for 24 hours were given as food for the experimental group. A control was also maintained and mortality was recorded every 12, 24, 48, 72 and 96 h.

Fecundity studies: Five sub-adult females and five adult males were introduced to each culture chamber for fecundity studies. Its fecundity was recorded in each oviposition by carefully separating eggs from the culture chamber using a fine brush. The number of eggs in each oviposition was counted. Five replicates were maintained for the study, and individuals were fed with jack leaves soaked in the sublethal concentration of cyfluthrin.

Probit analysis (Finney, 1971) was used to calculate LC50 and LC100, the sub lethal and safe concentrations of each cyfluthrin. Two-way ANOVA was conducted to find any difference between the number of eggs in different replicates and between different oviposition.

The results of toxicity studies of the pyrethroid pesticide cyfluthrin on the mortality of *C. javanus* at 5, 12, 14, 18, 20 and 22 ppm tested for different groups of 50 individuals for 96 hours indicated high mortality. The mortality rate at 5, 12, 14, 18, 20 and 22 ppm were 8.2, 12.1, 19.2, 25.6, 45.2, 47.3 percentage at 12 h; 10.1, 16.4, 20.8, 39.4, 61.1, 67.5 at 24 h; 12.8, 22.4, 29.2, 39.3, 68.4, 76.1 at 48 h; 13.9, 27.8, 35.4, 46.2, 76.2, 88.1 at 72 h and 15.8, 37.2, 52.9, 58.7, 91.3,100 at 96 h (Table 1). The results revealed that the mortality of *C. javanus* increases with the concentration of the insecticide cyfluthrin.

LC100 value for cyfluthrin was found to be 22.75 ppm at 96 h, 25.62 at 72 h, 28.55 at 48 h, 30.3 at 24 h and 40.32 at 12 h respectively. The LC50 value was noticed as 13.43 ppm at 96 h, 15.58 at 72 h, 17.2 at 48 h, 18.5 at 24 h and 23.26 at 12 h. The safe level concentration of cyfluthrin was calculated as 3.30 ppm and its sub lethal concentration was observed as 0.83 ppm (Table 3).

The average number of eggs laid by

C. javanus after the treatment of sub lethal concentration of cyfluthrin showed a drastic decline in the number of eggs laid in each oviposition. The number of oviposition remains the same as in control. The number of eggs laid was between 50 to 56 in the first oviposition, 59-74 in the second, 42-50 in the third, 43-54 in the fourth, 34-40 in the fifth and 22 to 32 in the sixth. The mean number of eggs laid in each oviposition ranged from 41.83 to 48.66. Two-way ANOVA results indicated that there is significant variation in the number of eggs laid in each replicate during different oviposition (P 0.00179; P<0.05) and between the number of eggs laid during different oviposition (P 3.43X10-13; P<0.05).

Table 1. Mortality of cyfluthrin on *C. javanus*

ppm	Mortality (%)				
	12h	24h	48h	72h	96h
5	8.2	10.1	12.8	13.9	15.8
12	12.1	16.4	22.4	27.8	37.2
14	19.2	20.8	29.2	35.4	52.9
18	25.6	39.4	39.3	46.2	58.7
20	45.2	61.1	68.4	76.2	91.3
22	47.3	67.5	76.1	88.1	100

Sub-lethal adverse studies of cyfluthrin on *C. javanus* showed that the average number of juveniles in insecticide-treated sets was 34.43 from the 45.93 eggs, and the number of exuvia was found to be 1.75. The hatching success rate was observed to be decreased to 74.9 per cent in treated groups, and its longevity was recorded to be significantly less in treated specimens when compared to the untreated groups (Table 2). Most organisms persist for about 90-110 days in normal conditions, and in cyfluthrin-treated groups, longevity was obtained to be approximately 50 days.

Collembola is a very primitive tiny insect that undergoes growth and moulting frequently throughout its life cycle. Adult female collembolans lay eggs for a long time in fresh, uncontaminated pollution-free soils. Collembola is known to be vulnerable to insecticides (Frampton, 1994). Species assemblages in polluted soils may change due to quantitative and qualitative changes in food, increased bioavailability of metals, avoidance of contamination by migration, and species-specific detoxification abilities (Liu *et al.*,2018). Emigration of collembola out of the insecticide plots may have contributed to the observed decline in density after insecticide application (Endlweber *et al.,* 2006). Ghosal and Hati (2019) observed no noticeable change in the collembolan population after the insecticidal application. Chronic toxicity of cadmium was more significant on life history parameters of ten days old *C. javanus* observed that mortality decreased by 62 per cent, moulting declined by 69 per cent, and fecundity decreased by 97 per cent (Sahana *et al*., 2014).

In the present study, life history parameters such as fecundity, number of eggs laid, number of juvenile emergences etc., are reduced in cyfluthrin treatment sets. According to Fountain and Hopkin (2004), the number of juveniles produced was positively related to the number of adult *Folosomia candida* that survived in the soil. Eijsackers (2009) reported a smaller life span, decreased fecundity and increased frequency of moulting due to the impact of herbicides 2, 4, 5-T on collembola. Similar results were obtained in the present investigation; the longevity of *C. javanus* was reduced to 50 days after being treated with cyfluthrin. An increase in the oxygen consumption rates of animals exposed to pesticides provides a clear indication of changes in metabolic activity (Mohammed *et al*., 1992). According to Saha and Joy (2014), the rates of moulting and fecundity are regarded as potential indices of the impact of xenobiotics in soil. Intoxication and intrusion of toxicants into the reproductive system may lead to the disruption of vital functions, and total disturbance of the reproductive hormones, thereby reducing fecundity. This follows earlier findings of Cardoso *et al.* (2014), who noticed that enhanced egg production

Table 2. Sub lethal effects of cyfluthrin on *Cyphoderus javanus*

Eggs laid (nos.)	Juveniles (nos.)	Exuvia (nos.)	Hatching success (%)	Longevity (in days)
45.93	34.43	1.75	74.9	10

Table 3. LC50, LC 100, safe and sub lethal concentrations of cyfluthrin on *Cyphoderus javanus*

LC50					LC100					Safe Conc (ppm)	Sub lethal Conc (ppm)
12h	24h	48h	72h	96h	12h	24h	48h	72h	96h		
23.26	18.5	17.2	15.58	13.43	40.32	30.3	28.55	25.62	22.75	3.30	0.83

was observed at different concentra-tions of insecticide carbaryl. The ageing time is a critical determinant of toxicity because it is directly related to the actual concentration to which soil organisms are exposed (Wee *et al.*, 2021). Pisa *et al.* (2015) reported that insecticides could significantly impact animal metabolism, affecting the detoxification, intermediary and energy metabolism pathways and reducing biomass gain. Dumestre *et al.* (1999) stated that elevated concentrations of copper in soils are toxic and may result in a range of effects, including reduced biological activity and subsequent loss of fertility.

From the experimental results, it is possible to conclude that the extensive utilization of the insecticide cyfluthrin negatively affects the fecundity, hatching, exuvium deposition and longevity of *C. javanus*. The greater rates of mortality in *C. javanus* due to cyfluthrin toxicity lead to the deterioration of the soil ecosystem and ecosystem balance.

ACKNOWLEDGEMENT

The authors are greatly indebted to DST-FIST for providing the necessary facilities in the research centre.

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(Received July 25, 2022; revised ms accepted October 16, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.804
ENTOMON 47(4): 469-472 (2022)
Short communication No. ent. 47417

Potential of resistance inducers for controlling *Agrotis segetum* Denis & Schiffermüller (Lepidoptera, Noctuidae) in sugar beet in Khuzestan, Iran

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ABSTRACT: Efficacy of some resistance inducers for reduction of *Agrotis segetum* (Lepidoptera, Noctuidae) in sugar beet was evaluated under field conditions. The inducers include salicylic acid, calcium silicate and sodium silicate which applied in two dosages, 100 and 50 per cent of recommended field dosages (RFD). The larval density in calcium silicate treatment was significantly lower than control (H"19.5%). However, other inducers, salicylic acid and sodium silicate, did not significantly affect the larval density. Reduction of the application dosage to 50 per cent RFD did not have significant effect on the inducer efficacy. © 2022 Association for Advancement of Entomology

KEYWORDS: Salicylic acid, calcium silicate; sodium silicate, cutworm

Sugar beet, *Beta vulgaris L.,* is attacked by many insect pests (Heibatian *et al.*, 2018). The black cutworm, *Agrotis segetum* Denis & Schiffermüller (Lepidoptera, Noctuidae) is a serious pest of sugar beet in many regions of Europe, Africa and Asia (Bowden *et al.*, 1987) including Iran (Darabian and Yarahmadi, 2017). The larvae of *A. segetum* consume the leaf epidermis, cuts seedling stems, and sometimes eats up the entire seedling through the stem at ground level (Heibatian *et al.*, 2018). There are many restrictions for chemical control of the pests in sugar beet fields (Darabian and Yarahmadi, 2017). Host plant resistance (HPR) considers as an appropriate alternative of chemical control in integrated pest management (IPM) programs (Mohammadi *et al.*, 2015a, b; Ongaratto *et al.*, 2021). Secondary metabolites (Zandi-Sohani *et al.*, 2018; Su *et al.*, 2018; Azadi *et al.*, 2018; Rajabpour *et al.*, 2019) and physical properties (Shahbi and Rajabpour, 2017; Kafeshani *et al.*, 2018) of the host plants can significantly affect the population density of pests. Fertilizers and plant hormone analogs play important role in the HPR enhancement (Abdollahi *et al.*, 2021). In this study, effects of silicon-based fertilizers and salicylic acid (as a plant hormone) in induction of HPR to *A. segetum* were investigated in sugar beet fields.

The commercial sugar beet cultivar, Antec® (Strube company, Germany), was cultivated (@900000 plants per ha) in a sugar beet field, 7000 m^2, in Amale seif country, Susa district, north Khuzestan province, southwest Iran (32°14'02"N; 48°14'15"E) during 2019-2020. The field divided was in 28 plots (each plot 200 m^2). The experiment was performed in a randomized complete block design with four replications (plots). In the sugar beet field, no insecticide was applied during the study. The

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Table 1. ANOVA parameters for main effects and interactions for *Agrotis segetum* density on sugar beet plants (data were $(X+1)^{0.5}$ transformed prior to analysis; error df=168)

Source	df	F value	P-value
Date	7	5.32	<0.0001
Resistance inducers*	3	1.86	0.0430
Application dosage	1	0.81	0.3690
Date × Resistance inducers	21	1.92	0.0125
Date × Application dosage	7	0.95	0.4676
Resistance inducers × Application dosage	2	2.01	0.1367
Date × Resistance inducers × Application dosage	14	1.44	0.1394

**(silicate potassium, silicate calcium and salicylic acid)*

experimental treatments include the different potential resistance inducers at 100 and 50 per cent recommended field dosage (RFD). The treatments were: silicate potassium at 100 per cent RFD (2 L ha^{-1}), silicate potassium at 50 percent RFD (2 L ha^{-1}), silicate calcium at 100 percent RFD (2 L ha^{-1}), silicate calcium at 50 percent RFD (1 L ha^{-1}), salicylic acid at 100 percent RFD (2.5 Mol L^{-1} ha^{-1}), salicylic acid at 50 percent RFD (1.25 Mol L^{-1} ha^{-1}). In control plots, the plants were sprayed with water. The plants were treated, single time, with a hand operated knapsack sprayer with hollow cone nozzle. The spraying was done at four leaf phenological stage of sugar beet plant, when the noctuid pests have occurred on sugar beet fields of Khuzestan province.

Sampling was weekly done from September 2019 to May 2020. At each sampling date, ten plants were randomly chosen by traveling in an X-shaped pattern through each plot and soil under each selected plant was removed, about 10 cm deep and wide, and the numbers of larva, 2^{nd}- 5^{th} instars, were counted. The factorial analysis (8 sampling dates × resistant inducer treatments × 2 application dosages) based on a completely randomized block design was carried out using the GLM procedure. The least significant difference (LSD) test, as a post hoc test of analyses of variance (ANOVA), was used for mean comparisons. The analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC).

The densities of the larvae in different sampling weeks were significantly different. There were treatment effects and their interactions on *A. segetum* density. The pest density in weeks 1-5 were significantly more than weeks 6-8 (Table 1, Fig. 1). Moreover, significant differences were observed in the larval infestations between different resistance inducer. Among the treatments, calcium silicate showed significant decrease in larval density of *A. segetum* in comparing with control (about 19.5% less than control). The results revealed that dosages (100 and 50% RFD) and its interactions have no significant effect on the larval density in sugar beet, indicating the lower dose is enough to lower the larval population (Table 1, Fig. 2).

Silicon depositions in host plant tissues provides a mechanical barrier against pest feeding. Furthermore, silicon is important element and elicitor for producing defensive metabolites, eg. tannins and phenolic compounds (Reynolds *et al.*, 2009). Induction of resistance using silicon-based fertilizers in corn to *Spodoptera frugiperda* Smith (Alvarenga *et al.*, 2017), sugarcane to *Sesamia* spp. (Nikpay *et al.*, 2015), and soybean to *Helicoverpa punctigera* Wallengren (Johnson *et al.*, 2020) were previously reported. The present study showed that salicylic acid has no significant effect in sugar beet

Fig. 1 Population density of *Agrotis segetum* larvae in different sampling weeks (Different letters indicate significant statistical difference at 5%)

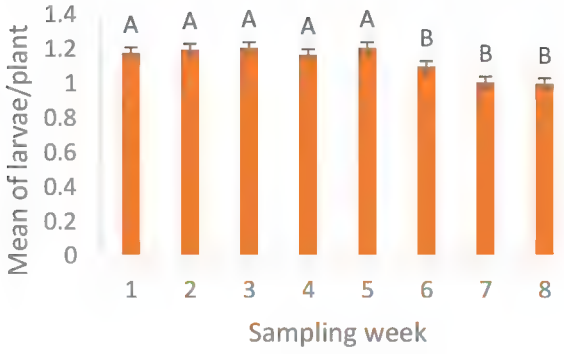
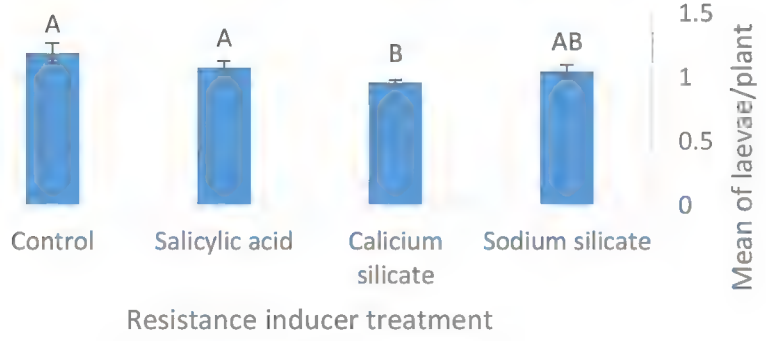


Fig 2. Population density of *Agrotis segetum* larvae in resistance inducer treatments (Different letters indicate significant statistical difference at 5%)



resistance to *A. segetum*. Salicylic acid as a phytohormone, mediates some multiple signaling pathways that involve in the plant defensive biochemistry. The significant effect of salicylic acid in HPR of many some noctuid pests including *H. agmigera* Hubner (War *et al.*, 2013) and *S. frugiperda* (Gordy *et al.*, 2015) were documented. Different feeding behavior of *A. segetum* may be the main reason of the conflict results. The noctuid pests, *H. agmigera* and *S. frugiperda* feed on leaf tissues. It is demonstrated that salicylic acid causes induction of defensive chemicals, especially poly phenols, and enzymes, including jasmonic acid and polyphenol oxidase, in host plant leaves (Abdollahi *et al.*, 2021). In conclusion, calcium silicate (1 L ha^{-1}) can be used for resistance induction of sugar beet to *A. segetum*. The resistance can be integrated in IPM programs of sugar beet fields for sustainable control of the pest.

ACKNOWLEDGEMENT

The research was financially supported by Agricultural Sciences and Natural Resources University of Khuzestan, Iran.

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(Received August 30, 2022; revised ms accepted November 12, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.805
ENTOMON 47(4): 473-476 (2022)
Short communication No. ent. 47418

Chemical characterization of n-alkane compounds in the leaves of *Holoptelea integrifolia* and its repellence against Japanese encephalitis vector

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ABSTRACT: Epicuticular wax extract bearing n-alkane compounds were isolated from leaves of *Holoptelea integrifolia* and its chemical characterization was done by GC-MS analysis. Seven n-alkane compounds were isolated from epicuticular wax of *H. integrifolia,* which are Undecane [$C_{11}H_{24}$], Decane 5-methyl- [$C_{11}H_{24}$], Dodecane [$C_{12}H_{26}$], Undecane, 3,6-dimethyl- [$C_{12}H_{26}$], Hexadecane, 2,6,10,14-tetramethyl- [$C_{20}H_{42}$], Tridecane [$C_{13}H_{28}$], and Tetradecane [$C_{14}H_{30}$]. Different concentrations of crude extract as well as epicuticular wax extract bearing n-alkane each @ 2, 4 and 5 ppm cm^{-2} applied on human hand surface for repellence against *Culex vishnui* (vectors of JE) and at different time of exposure, gave a maximum protection of 73.33 per cent in the case of crude extract, and 94.33 per cent with epicuticular wax extract, both at 5 ppm cm^{-2}, up to five hours of exposure.

KEYWORDS: Indian Elm tree, epicuticular wax extract, repellent, *Culex vishnui* group

Mosquito at the time of blood feeding transmits extremely harmful pathogens from host to host causing malaria, yellow fever, dengue, zika, filariasis, and Japanese encephalitis (JE). Female mosquito uses blood meal as protein and vitamin source for egg development and blood proteins are used as building blocks for the synthesis of egg yolk proteins. The first major epidemic of JE in India was reported from Bankura and Burdwan districts of West Bengal in 1973 (*Curic et al., 2014),* caused by the mosquito borne JE virus (Mahmud *et al.*, 2010). According to WHO (1981) more than 3 billion people of South-East Asia and Western Pacific regions are under the risk of JE transmission. Extracts of different parts of several plants have been reported earlier as mosquito repellent along with others activities (Adhikari *et al.,* 2012; Rawani *et al.,* 2012; Adhikari and Chandra, 2014; Bhattacharya and Chandra, 2014; Haldar *et al.,* 2014).

Epicuticular wax on the surface of plant leaves and other parts of the plant plays an important ecological role in interaction with insects as attractant or deterrent (Muller, 2006). *Holoptelea integrifolia* (Roxb.) belonging to the family Ulmaceae and commonly known as the Indian Elm tree, is found all over the Indian peninsula (Mahmud *et al.,* 2010). From ancient times, this tree was well known due to its medical importance. Traditionally different parts of this plant were used for the treatment of different diseases like inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound

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healing, leprosy, diabetes, dysmenorrhoea and rheumatism (Kumar *et al.,* 2011). According to Singha *et al.* (2012) acetone extract of leaf of *H. integrifolia* have very good larvicidal activity on *Cx. vishnui* group of mosquitoes. Antibacterial efficacy was also established from different solvent extracts of leaf of *H. integrifolia* against beta-lactam resistant strain of *Staphylococcus aureus* but diethyl ether extract with 1, 4-naphthalenedione as the bioactive principle was found to perform the best result (Vinod *et al.,* 2010). Epicuticular wax of leaf of this plant bears a mixture of different straight or branched chain alkane, esters, aldehydes, alcohols, and fatty acids, among others (Kunst and Samuels, 2003; Koch and Ensikat, 2008), but alkanes play vital role in plant-insect interaction (Müller, 2005; 2006).

Present study was aimed at isolating n-alkane compounds from epicuticular wax of leaf of *H. integrifolia* by application of non-polar solvent (n-hexane) and characterization of n-alkane compounds by GC-MS analysis as well as to establish their role as mosquito repellent on adult *Cx. vishnui* group of mosquitoes.

Fresh and mature leaves of *H. integrifolia* were harvested randomly from plants growing at the outskirts of Dedipur, Burdwan, India. Collected mature leaves were initially rinsed in tap water followed by distilled water to remove dust, unwanted debris etc. and dried on a paper towel. Crude leaf extract was prepared by mortar and pestle and the extract was allowed to become a semisolid paste through simple air drying. Fifty grams of leaves were dipped in two litre of cold n-hexane solution for extraction of epi-cuticular wax at room temperature for 45 min. The extract was filtered through Whatman no.41 filter paper. The filtrate was dried in rotary evaporator. Thin layer chromatography (TLC) was done for fractionation of extract, where carbon tetrachloride was used as mobile phase. Calculated R_f value from TLC was 0.68. The TLC fractions with same Rf value (Rf value = 0.68) were scraped and collected from 40 TLC plates. TLC plates (thickness of 0.5 mm) were prepared with silica gel G (Merck, Mumbai, India) using a Unoplan coating apparatus (Shandon, London, UK) (Bhattacharjee *et al.,* 2010).

Larvae of *Cx. vishnui* group of mosquitoes were collected from rice fields surrounding Golapbag campus, The University of Burdwan. Larvae were reared in plastic tray till they transformed into the pupa. Pupae were isolated manually by dropper and transferred to mosquito cage to metamorphose into adult form. Blood starved adult female mosquitoes were used for the repellence test. Repellence activity of crude extract and epicuticular wax of leaf of *H. integrifolia* was studied separately on human volunteers following WHO (2009) protocol. Required approval was obtained from the Institutional Ethics Committee (IEC/BU/2021/3, dt-24/6/21).

Three to four day old blood starved 100 adult female mosquitoes were kept in a net cage. Repellence test was conducted in a cage measuring 70 x 60 x 30 cm^3. Isopropanol was used for cleaning the arms of the volunteer. After air-drying 25 cm^2 area of the skin surface on each arm was exposed, remaining areas were being covered by rubber gloves. 2 ppm cm^{-2}, 4 ppm cm^{-2} and 5 ppm cm^{-2} concentrations of each of the crude extract and epicuticular wax of leaf of *H. integrifolia* were applied on the exposed area of the experimental arm. On the control arm, respective extracts were not applied before exposing to starved mosquitoes in the cage. The numbers of bites were recorded over 5 minutes after every 60 min, from 0.00 h to 5.00 h. Each experiment was repeated three times. Percentage of protection from mosquito bite was measured by using the following formula:

$$\text{Protection (\%)} = \frac{\text{Number of bites received by control arm - Number of bites receieved by treated arm}}{\text{Number of bites received by control arm}} \text{ X } 100$$

Characterization of epicuticular wax was done by GC-MS analyses, following NIST (National Institute of Standards and Technology) Library. One il sample was injected in split mode in the instrument (GCMS-QP2010 plus). During sample injection the initial temperature was set at 60 ^{0}C and the temperature was increased to 270 ^{0}C in a successive manner. In the whole procedure Helium was used as carrier. Mass spectral analysed data

were recorded with 40-650 m/z scanning range and with speed of 5 scan sec^{-1}. Statistical analyses of collected data were done through Microsoft Excel software.

In the repellence test the highest efficacy of protection for 5 hours, was recorded at 5 ppm cm^{-2} with the epicuticular wax extract bearing n-alkane compounds (94.33 %) and in the crude extract (73.33%). The mean repellency potential varied significantly with crude extract and epicuticular wax extract on *Cx. vishnui* group and again with different duration of exposure and at different concentrations (Table 1). Further observations revealed that the potentiality of the crude extract and epicuticular wax extract gradually decreased after five hours and the activity persisted up to 8 h.

Table 1. Repellence of crude extract and epicuticular wax extract on *Culex vishnui* mosquito

Conc. (ppm cm^{-2})	Duration of exposure (h)	Protection (Mean ±SE) (%)	
		Crude	Epicuticular wax
2	3	37.67±0.33	64.67±0.67**
4	4	63.33±0.33	82.67±0.67**
5	5	73.33±0.33	94.33±0.33**

** *Highly significant; (Mean ±SE)*

GC-MS analysis of n-alkane compounds isolated and identified were as follows: Undecane [C11H24], Decane 5-methyl- [C11H24], Dodecane [C12H26], Undecane, 3,6-dimethyl- [C12H26], Hexadecane, 2,6,10,14-tetramethyl- [C20H42], Tridecane [C13H28], and Tetradecane [C14H30]. Out of isolated seven compounds, dodecone, undecane and tridecane are present as major constituents in epicuticular wax from leaf of *H. integrifolia*. Of these compounds, only dodecane and tetradecane were previously reported for their mosquito repellence and others were not reported for their mosquito repellence (Pojmanova *et al.,* 2019; El-Sayed, 2020; Lu *et al.,* 2020; Sutthanont *et al.,* 2010). In epicuticular wax of plants, the major components are n-alkanes, which are mainly ranging from C12 to C27 in carbon chain lengths. Epicuticular wax has many physiological functions, including protection against UV light and moderation of gas exchange through stomata (Bhattacharjee et al. 2010). Interaction between plant-insect and plant-pathogen were mainly triggered by epicuticular wax components of plant parts (Müller, 2006; Carver and Gurr, 2006). Leaves of different plant species contain different n-alkane profile (Barik et al., 2004; Jetter and Schäffer, 2001).

Isolated epi-cuticular wax from leaf of *H. integrifolia* has repellent potentiality against JE vector, *Cx. vishnui.* It shows repellence at a very low concentration for a longer time. Both crude and epi-cuticular wax bearing n-alkane compounds are safe for use on skin surface, as the application has not shown any discomfort or reaction. It may be a better alternative against commercially available different mosquito repellents.

ACKNOWLEDGEMENTS

Authors are thankful to DST-FIST and SAP-DRS for providing instrument facilities in the Department.

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(Received August 30, 2022; revised ms accepted November 12, 2022; published December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.806
ENTOMON 47(4): 477-482 (2022)
Short communication No. ent. 47419

Growth and development of *Amrasca biguttula biguttula* Ishida (Hemeptera, Cicadellidae) during different seasons on okra

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ABSTRACT: The influence of three seasons namely pre - kharif (Feb-May), kharif (May-Aug) and post-kharif (Aug-Nov) on the biology of jassid on okra under natural climatic conditions revealed that the developmental periods showed differences over seasons. The total nymphal period was longest in post-kharif (8.90±0.91days) followed pre-kharif (7.15±0.75 days) and the shortest during kharif (6.60±0.52 days). The longest total life span was observed in post-kharif (38.29±2.79) followed by pre-kharif (34.90±1.47 days) and shortest during kharif (33.75±1.89 days). Maximum eggs was laid in post-kharif (18.70±2.45 eggs/ female), followed by the pre-kharif (17.20±1.62 eggs/female) and least in kharif (16.20±1.55 eggs/ female). © 2022 Association for Advancement of Entomology

KEYWORDS: Biology, growth stages, pre-kharif, kharif, post-kharif, variation

Abelmoschus esculentus L. (Moench) is an important vegetable crop grown in tropical and sub-tropical parts of the world. Okra has occupied a prominent position among the export-oriented vegetables in India because of its high nutritive value, palatability and good post-harvest life. Among different insect pests infesting okra in terai region of West Bengal, fruit borer and jassid are key pests and considered as limiting factors in productivity of the crop okra. Jassid *Amrasca biguttula biguttula* Ishida (Homoptera, Cicadellidae), is a polyphagous pest and causes considerable damage to wide range of crops. The nymphs and adults suck the plant sap mainly from the lower surface of leaves and cause phytotoxic symptoms known as hopper burn which results in complete drying of leaves (Jayasimha *et al.,* 2012). In depth study of biology of this pest was attempted with sole motive to study the variation of growth stages in three different seasons.

The biology of *A. biguttula biguttula* was studied in instructional farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, India. The field collected final 30 instar nymphs of *A. biguttula biguttula* were released in potted Arka anemika variety of okra plants covered with net. The final instar nymphs were identified based upon the extent of wing pads developed and were maintained in rearing cages till they reach adult

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stage and lay eggs. After egg laying, the adults were removed. Observations were recorded for the incubation period and nymphal period. Ten pairs of newly emerged nymphs were collected and released on potted okra plant kept inside the cage size of 1 X 1m^2 wide with 1m height for eggs laying in five replications. The nymphs were observed at intervals of eight hours. The time of moulting was recorded when the exuviae were observed. The newly hatched nymph was considered the first instars and after moulting the nymphs were considered the second instars and so on. The number of instars and days required for each instar were recorded based on the moulted skin and size. The male and female sexes were identified based on the prominent adeagus in male and genetalia in female (Thirumalaraju, 1984). They were closely observed for matting and allowed egg laying. Cage was opened daily and the leaves were observed under magnifying glass for oviposition and this was continued till the last egg was laid. The duration of pre-oviposition, oviposition and post-oviposition were recorded.

Thirty newly emerged adults were transferred to fresh caged potted okra plant in 2:1 male-female sex ratio and allowed to mate. Each of the female was counted as a replication. Cage was opened daily and the leaves and fruits were observed under the magnifying lens to know the oviposition and this was continued till the last egg was laid. The sexed females laid translucent slightly oval shaped eggs scattered under the surface of the leaves of okra. In occasional case eggs were also laid on upper surface of leaves. The pre-oviposition, oviposition and post-oviposition duration were recorded. The total number of eggs laid by per female was recorded. Observations were recorded on incubation period, nymphal period and adult longevity. The time elapsed between the emergence of each individual and its death was recorded as longevity. The overall developmental duration from egg to adult were calculated for both male and female. The duration of each generation was estimated on the basis of the average length of the life cycle. SAS software (ver. 9.2) was used for data analysis. One way ANOVA was performed for each of the parameters and separation of the means was done using the Least Significant Difference test.

The overall developmental duration from egg to adult as well as the fecundity varied significantly over the seasons (Table 1).

Incubation period: The maximum duration of incubation period was found during post-kharif (9.10±0.88 days) followed by the pre-kharif (6.80±0.79 days) and kharif (6.60±0.70 days). The average incubation period of three seasons was 7.50±1.35 days.

Nymphal period: The jassid underwent through 5 nymphal instars before reaching the adult stage. The duration of each instar varied over the three different cropping seasons. The first instar nymph was longest in kharif (1.40±0.62) and pre-kharif (1.40±0.52) and shortest in post-kharif (1.50±0.53) with an average of 1.43±0.06. The development time of second instar nymph was longest in kharif (1.20±0.35) and pre-kharif (1.20±0.55) and shortest in post-kharif (1.55±0.44) with an average of 1.32±0.20. The third instar nymph took longest time in post-kharif (1.45±0.44a) and shortest during kharif (1.10±0.21) and pre-kharif (1.15±0.24) with an average of 1.23±0.19. The duration of fourth instar nymph was longest in post-kharif (2.00±0.47) followed by pre-kharif (1.60±0.39) and shortest in kharif (1.30±0.48). The longest development period of fifth instar nymph was recorded in post-kharif (2.40±0.52) followed by pre-kharif (1.85±0.24) and shortest in kharif (1.55±0.37) with an average 1.93±0.43 days. The total nymphal period was found longest in post-kharif (8.90±0.91days) followed pre-kharif (7.15±0.75 days) and the shortest during kharif (6.60±0.52 days). The total average nymphal period was 7.55±1.20 days.

Adult stage: In general, the females lived longer than the males. The females lived longer in the pre-kharif period (24.94±2.58), followed by the post-kharif period (24.55±1.19) and shorter in the kharif period (22.75±1.70). The longest male longevity was recorded in post-kharif period (22.65±1.29), followed by pre-kharif period (21.80±2.20) and shortest in kharif period (20.07±2.23). The mean

Table 1. Duration of developmental stages of *Amrasca biguttula biguttula* over seasons

	Pre-kharif	*Kharif*	*Post-kharif*	
Mean temperature (Min-max)	26.07°C (20.60-31.55 °C)	28.08 °C (23.65-32.50 °C)	27.37 °C (21.92-32.82 °C)	
Mean RH (Min-Max)	75.29% (71.97-78.61 %)	83.26% (78.69-87.83 %)	78.45% (74.73-82.16%)	
Developmental stages	Duration in days (Mean±SD)			
	Pre-kharif	Kharif	Post-kharif	Average
Incubation Period	6.80±0.79	6.60±0.70	9.10±0.88	7.50±1.35
Nymphal Period				
1st Instar	1.40±0.52a	1.40±0.62a	1.50±0.53a	1.43±0.06
2nd Instar	1.20±0.35a	1.20±0.55a	1.55±0.44a	1.32±0.20
3rd Instar	1.10±0.21b	1.15±0.24b	1.45±0.44a	1.23±0.19
4th Instar	1.60±0.39b	1.30±0.48b	2.00±0.47a	1.63±0.35
5th Instar	1.85±0.24b	1.55±0.37b	2.40±0.52a	1.93±0.43
Total	7.15±0.75b	6.60±0.52c	8.90±0.91a	7.55±1.20
Male longevity	21.80±2.20b	20.07±2.23c	22.65±1.29a	21.51±1.23
Female longevity	24.55±1.19a	22.75±1.70b	24.94±2.58a	24.08±1.56
Life cycle	34.90±1.47b	33.75±1.89b	38.29±2.79a	35.65±2.36
Fecundity	17.20±1.62b	16.20±1.55b	18.70±2.45a	17.37±2.23
Pre-oviposition	3.65±0.41a	3.45±0.44a	2.85±0.58b	3.32±0.42
Oviposition	17.30±1.34b	16.10±1.60c	18.44±2.28a	17.28±1.56
Post Oviposition	3.60±0.46a	3.20±0.42a	3.65±0.47a	3.48±0.19

Note: Within row means followed by the same letter(s) are not significantly different at 5% level

male and female adult life expectancy was found to be 21.51±1.23 and 24.08±1.56 respectively.

Life cycle: The number of eggs laid was higher in post-kharif (18.70±2.45 eggs/female), followed by pre-kharif (17.20±1.62 eggs/female) and the least number of eggs was laid in kharif (16.20±1.55 eggs/female). The average egg laying time of the three seasons was reported to be 17.37±2.23 days.

Fecundity: The number of eggs was higher in post-kharif (18.70±2.45 eggs/female) followed by the pre-kharif (17.20±1.62 eggs/female) and least number of eggs was laid in kharif (16.20±1.55 eggs/female). The average eggs of three seasons were recorded as 17.37±2.23 days.

Duration of oviposition: The period oviposition varied significantly. The longest pre oviposition was in pre-kharif (3.65±0.41days) followed by kharif (3.45±0.44) and shortest in post-kharif (2.85±0.58) with an average of 3.32±0.42. The longest oviposition period was recorded during post-kharif (18.44±2.28) followed pre-kharif (17.30±1.34) and shortest in kharif (16.10±1.60) with an average of 17.28±1.56. Post-oviposition period in post-kharif

was 3.65±0.47, followed by pre- kharif (3.60±0.46) and shortest during kharif (3.20±0.42) with an average of 3.48±0.19 (Table 1).

The incubation period of *A. biguttulla buguttulla* was recorded as 10 days during winter by Afzal and Ghani (1953) which confirms the post-kharif incubation. The incubation period of pre kharif and kharif recorded in the present study was supported by Rao (2003), Shivanna *et al.* (2009), Jayasimha *et al.* (2012), Jayarao *et al.* (2015), Kumar and Bhat (2012) and Shreevani *et al.* (2013). In the present observation, the average incubation period was obtained as 7.50±1.35. The results is consistent with Bhalani and Patel (1981) (7.00 days); Sharma and Sharma (1997) (7.30 days); 7.41±0.48 days (Jayasimha *et al.*, 2012) and Jayarao *et al.* (2015) (8.04±0.51 days). While shorter incubation of 4.50-5.30 days reported by Singh (1976) contradicts present incubation period. On other hand the longer duration were reported by, Shivanna *et al.* (2009) (11.68±3.74 days); Kumar and Bhat (2012) (16.9 to 17.6 days) and Shreevani *et al.* (2013) (12.30±2.42 days). Jayasimha *et al.* (2012) reported duration of different nymphal instar which is in accordance with the present study. The different nymphal period of post-kharif was in agreement with Jayarao *et al.* (2015). Shivanna *et al.* (2009) and Shreevani *et al.* (2013) reported longer duration of each nymphal period.

Jayasimha *et al.* (2012) reported the male and female longevity of 22.85±1.87 and 26.66 ±1.92 days respectively. This confirms the male and female longevity of post-kharif period. The 21 days of male longevity confirm the male longevity of pre-kharif but female longevity of 28 contradicts the female longevity of pre-kharif period (Kumar and Bhat, 2012). However Jayarao *et al.* (2015) reported shorter male and female longevity of 16 and 18 days. Jayasimha *et al.* (2012) observed that the pre-oviposition, oviposition and post-oviposition periods of 3.52±0.34, 16.54±0.37 and 3.85±0.24 days, which confirm the present pre-oviposition, oviposition and post-oviposition periods of pre-kharif and post-kharif. The pre- oviposition and post-oviposition period was also in line with the findings of Jayarao *et al.* (2015) and Shivanna *et al.* (2009); however shorter oviposition period of 6.65±0.26 and 3.90 days were recorded by the above workers respectively. The life cycle of pre-kharif, kharif and post-kharif was in close agreement to Jayasimha *et al.* (2012) and Sharma and Sharma (1997) as they observed life cycle of 30.31±2.07 days and 33.70 days respectively. However Shivanna et al. (2009) and Jayarao *et al.* (2015) reported shorter length of life cycle of 27.63 days and 29.50±1.96 days respectively.

The fecundity was found within the range of 16.20-18.70 in present study. The similar fecundity of 14.00 to 20.00 with an average of 16.60 ±1.98 eggs per female was also reported by the Jayasimha *et al.*, (2012). Sharma and Sharma (1997) reported an average of 17.55 eggs per female with an average of 17.35. Jayarao *et al.* (2015) also obtained total fecundity of 17.53 ± 0.52 per female and Sharma and Sharma (1997) recorded the fecundity as 17.20 and 17.50 eggs. This confirms that the present fecundity of all the three seasons.

During the pre-kharif season the Jassid completed its life cycle with a shorter period of 34.90 days, suggesting a greater number of generations. Moreover, the fecundity of 17.20±1.62 in the pre-kharif season may lead to high population formation. On the other hand, in the kharif season, the shorter life cycle of 33.75 days indicates a higher number of generations, but the lower fecundity of 16.20 eggs/female and the heavy rains may prevent the jassid population from reaching a higher level. The highest fecundity was recorded in post-kharif (18.70±2.45). However, the longest life cycle of 38.29±2.79 may result in a lower number of jassid. The above information will be useful in the integrated pest management.

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(Received July 20, 2022; revised ms accepted October 13, 2022; printed December 31, 2022)

https://doi.org/10.33307/entomon.v47i4.807
ENTOMON 47(4): 483-486 (2022)
Short communication No. ent. 47420

The record of the Jewel beetle, *Strigoptera bimaculata* (L., 1758) (Coleoptera, Buprestidae) from India

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ABSTRACT: *Strigoptera bimaculata* (L., 1758) is a tropical Buprestid (jewel beetles) found throughout Southeast Asia to Northern Australia. Adult *S. bimaculata* was observed in The American College in Madurai, Tamil Nadu, India. The present record extends its known distribution range further towards the west being the western most occurrence of this species in the world.

KEYWORDS: Jewel beetle, occurrence, distribution range

The order Coleoptera is represented by 3,44,105 species worldwide among them 17,036 species are found in India (Kriti and Sidhu, 2015). The family Buprestidae includes 775 genera with 15,500 species of beetles, making this a largest group of beetles known (Bellamy, 1985; Holynski, 1993; Bily, 2002). The family represents all the jewel beetle species with metallic glossy iridescent colours. The jewel beetle, *Strigoptera bimaculata* (L., 1758) (Coleoptera: Buprestidae) is a tropical and distinctive metallic blue buprestid found throughout Southern East Asia to Northern Australia. Though it is widely distributed very little is known about its global distribution and biology. All the known previous records made the species confined to a cluster of few countries in proximity.

An adult *S. bimaculata* was observed in the campus of The American College in Madurai, Tamil Nadu, India (Fig. 1). It was observed for an hour rather no feeding behaviour or any other specialized behaviour was noted. Photographic documentation was done using (Samsung M51) and identified up to species level on the basis of illustration and descriptions from the literature (Ek-Amnuay, 2008; Hawkeswood, *et al.,* 2018; Hawkeswood, 2021). The Map illustrating the global distribution of *Strigoptera bimaculata* was constructed using Quantum Geographical Information System (QGIS Desktop 3.16.15).

Global Distribution of *Strigoptera bimaculata* with past and present records were represented (Table1, Fig. 2).

This seems to be the first published record of *S. bimaculata* in India. According to the past observations and records *S. bimaculata* has been reported from Thailand, Vietnam, Cambodia, Myanmar, Indonesia and Australia. Hawkeswood

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Fig. 1 *Strigoptera bimaculata* (Linnaeus., 1758) (Coleoptera: Buprestidae) found from, Madurai, Tamil Nadu, India

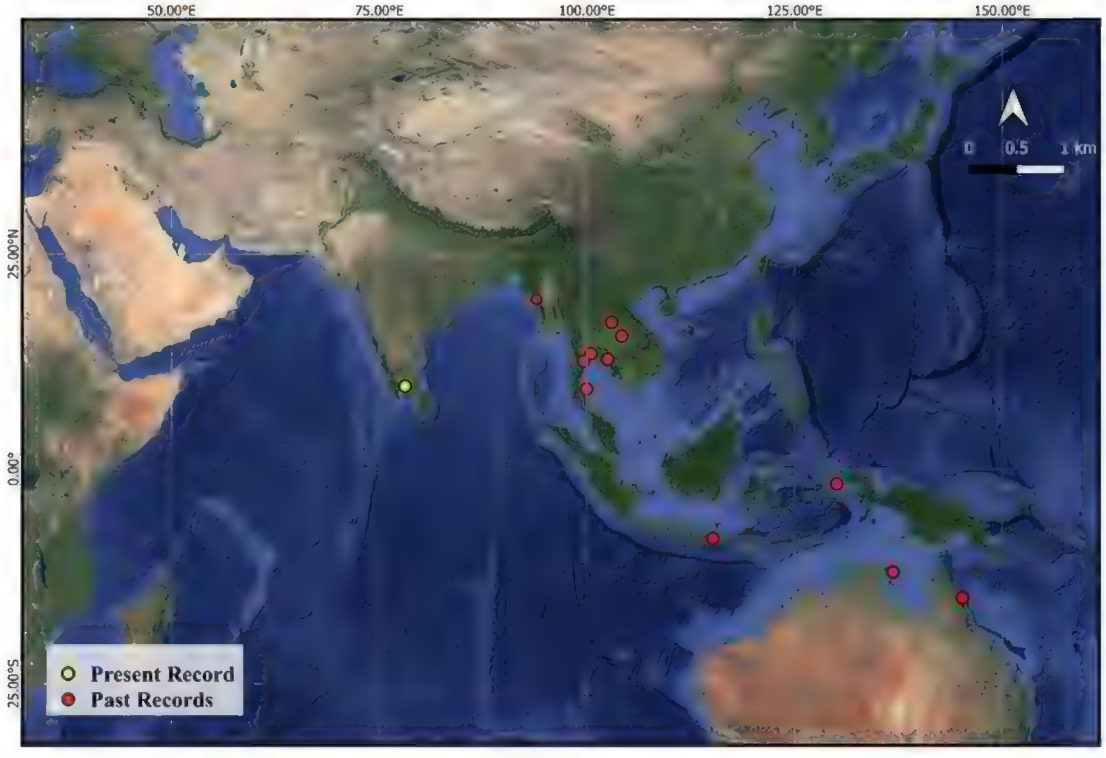

Fig. 2 Map illustrating the global distribution of *Strigoptera bimaculata* with previous records (red dots) and the present record in India (yellow dots)

Table 1. List of past and present record of adult *Strigoptera bimaculata* in the world

No.	Country	Catal.no.	Year of Publication	Authors	Reference
1	Indo-Australia	BE.2277284	2017	Creuwels. J	Naturalis Biodiversity Center
2	Vietnam	BA03134	2020	Sawada. Y & Kurubi. K	Osaka Prefectural Minoh Park Insects Museum. National Museum of Nature and Science, Japan.
3	Australia	K.91150	2021	Chaseling. L	Australian Museum
4	Australia	BUP207	2021	-	Museums Victoria
5	Australia	COL78838	2021	-	Museums Victoria
6	Thailand	8912826	2022	Elliot Greiner & Stephen Gottwald	iNaturalist
7	Thailand	21068258	2022	Les Day	iNaturalist
8	Indonesia	107697247	2022	Mehd Halaouate & Stephen Gottwald	iNaturalist
9	Thailand	48765883	2022	Didier Levasseur & Stephen Gottwald	iNaturalist
10	Thailand	79742338	2022	Ian_dugdale	iNaturalist
11	Thailand	90627712	2022	Tanapong	iNaturalist
12	Indonesia	97736793	2022	Stephen Gottwald	iNaturalist
13	Cambodia	117433972	2022	Mark Spicer	iNaturalist
14	India	126925904	2022	Kishore R	iNaturalist
15	Myanmar	121596203	2022	Frankthierfelder	iNaturalist
16	China	156953	2022	Fagerstrom. C	Lund University Biological Museum Insect Collections Inventory.
17	Indonesia	193201	2022		Sys Tax -Zoological Collections
18	Australia	196056	2022		Sys Tax -Zoological Collections
19	Philippines	197650	2022		Sys Tax -Zoological Collections

(pers. comment) stated the past record of this species from India is unknown due to lack of proper references. When re-examined these references provided in Ek-Amnuay (2008) about its distribution in India, there was no evidence of its occurrence. It may probably present in India and Malaysia although conclusive evidence is required.

Though *S. bimaculata* has been recorded from seven countries, most of its sightings were from mangroves and its adjacent mainland. Hawkeswood (1986, 1988) recorded the beetle breeding in mangroves *Ceriops tagal* (Pers.) C.B. Rob. (Rhizophoraceae) and *Camptostemon schultzii* Mast. (Malvaceae) in Australia. The present record of this species in Madurai is approximately 200 km far from the Gulf of Mannar National Park. The National Park has mangroves with rich tree diversity and it is one of the remnant patches of Indian Mangrove *Ceriops tagal* (host plant of *S.bimaculata*) along the Indian coast (Selvam *et al.,* 2004). *S. bimaculata* appears to breed in the trunks (or major branches) of mature trees of *Hevea brasiliensis* Muell. Arg. (Euphorbiaceae) rubber trees (Hawkeswood, 2021). The past records also depict similar pattern of its occurrence in mainland far from its host plant in the mangroves.

The present record extends its known distribution range further towards the west being the western most occurrence of this species in the world. Furthermore, studies should be carried out to reveal its biology, distribution and host interactions over a period of time.

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(Received August 30, 2022; revised ms accepted November11, 2022; printed December 31, 2022)

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ACKNOWLEDGEMENTS

The EDITORIAL BOARD of ENTOMON profoundly express their profuse and sincere thanks and gratitude to the following peer reviewers for sparing their valuable time in critically going through the manuscripts and in giving constructive comments and suggestions on the articles published during 2022 in the ENTOMON volume 47, issues 1 to 4.

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The ENTOMON is grateful to the following NAAS members for recommending the ENTOMON, for the NAAS scoring of scientific journals 2021 by the NASC, New Delhi.

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AUTHOUR INDEX

Statement of ownership and other particulars of ENTOMON

(Form IV, Rule 8 of Registration of Newspapers (Central) Rules 1956)

1.	Place of publication	:	Trivandrum
2.	Periodicity of publication		Quarterly
3.	Printer's name, nationality and address	:	Dr K D Prathapan, Indian, Secretary, Association for Advancement of Entomology, Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellayani PO, Thiruvananthapuram 695522, Kerala, India
4.	Publisher's name, nationality and address	:	- do-
5.	Editor's name, nationality and address	:	Dr M S Palaniswami, Indian, Chief Editor, ENTOMON, Association for Advancement of Entomology, Thiruvananthapuram 695522, Kerala, India
6.	Name and address of the Individual who owns the paper	:	Association for Advancement of Entomology, Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellayani PO, Thiruvananthapuram 695522, Kerala, India

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31 December 2022 — Publisher, ENTOMON

Association for Advancement of Entomology

(Reg. No. 146/ 1975)

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EXECUTIVE COMMITTEE (2022 – 2025)

Reg. No. 123SBN

Published by :
Association for Advancement of Entomology
Email : aae@kau.in; web: www.entomon.in

Layout and printing at SB Press, Trivandrum - 695 001, Kerala, India
Ph : 0471-4000645, e-mail : sbpress.tvm@gmail.com